Universidade de Aveiro Departamento de Biologia
Ano 2017

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A resposta funcional de plantas à desertificação e degradação do solo – contributo para estratégias de restauro

Plant functional response to desertification and land degradation – contribution to restoration strategies

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia e Ecologia das Alterações Globais, realizada sob a orientação científica do Doutor Amadeu M. V. M. Soares, Professor Catedrático do Departamento de Biologia da Universidade de Aveiro, da Doutora Cristina Branquinho, Professora Associada c/ Agregação da Faculdade de Ciências da Universidade de Lisboa e da Doutora Otília Correia, Professora Associada c/ Agregação da Faculdade de Ciências da Universidade de Lisboa

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Ao Tiago e à Carolina, aos meus pais.

o júri

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palavras-chave alterações climáticas; ecologia de zonas áridas; diversidade funcional; restauro

resumo

É crucial entender o impacto das alterações climáticas nos ecossistemas áridos, pois estas poderão agravar a desertificação e degradação dos solos, comprometendo o funcionamento dos ecossistemas e os serviços a eles associados. Os atributos funcionais ligam as espécies aos processos do ecossistema, fornecendo um entendimento mecanicista da sua resposta ao clima. O principal objectivo desta tese foi modelar a resposta de ecossistemas áridos ao clima com base em atributos funcionais de plantas (AFP), usando um gradiente climático espacial para prever alterações no tempo. Pretendeu-se assim selecionar um indicador baseado em AFP para monitorizar os efeitos do clima, e contribuir para melhorar as estratégias de gestão e restauro de zonas áridas. Embora a maioria das métricas de diversidade funcional requeiram a quantificação dos AFP no campo, não existia consenso sobre qual o melhor método para ser usado à escala global. Comparámos diferentes métodos, e demonstrámos as vantagens do método dos quadrados pontuais na monitorização de fina-escala dos AFP em zonas áridas. Desconhecia-se quais os principais AFP que respondiam à aridez, o que é essencial para o seu uso como indicadores de alterações no ecossistema. Identificámos nove AFP que respondem à aridez. Esta afectou a respectiva média, e reduziu a diversidade funcional. O uso de gradientes climáticos no espaço para prever alterações no tempo, requer a comparação de ambos os padrões, para a respectiva validação. Verificámos que as variações climáticas entre anos afectam os AFP, indicando que as mudanças funcionais são transitórias. Contudo, a diversidade funcional diminuiu em condições climáticas mais limitantes. Assim, face a uma maior aridez, espera-se que os padrões de resposta no tempo convirjam para os verificados no espaço. Os AFP podem responder de forma mais previsível ao clima do que a diversidade de espécies. Desenvolvemos um indicador de diversidade funcional baseado em múltiplos AFP, que diminuiu de forma monotónica não-linear com a aridez, mostrando ter maior capacidade preditora da resposta ao clima do que a diversidade de espécies. Os factores biofísicos locais modulam o efeito do clima na vegetação. Verificámos que os factores topo-edáficos têm um papel chave nos AFP envolvidos na colonização por arbustos. Os factores climáticos pouco influíram, sugerindo que as alterações climáticas não promoverão a colonização por arbustos. Os AFP podem ser uma ferramenta essencial no restauro de zonas áridas. Apresentámos uma visão geral sobre projectos de restauro, mostrando a necessidade de uma melhor integração dos AFP no restauro de zonas áridas. Ao demonstrar que os AFP são indicadores consistentes do impacto do clima nos ecossistemas, contribuímos para melhorar as previsões dos efeitos das alterações climáticas nas zonas áridas, e desenvolvemos um indicador que pode ser usado para mapear áreas em risco de desertificação e degradação do solo, potencialmente a uma escala global.

keywords climate change; dryland ecology; functional diversity; restoration

abstract

It is crucial to anticipate the impacts of climate change on drylands, as it may aggravate desertification and land degradation, hampering ecosystems functioning and associated services. Functional traits determine species' responses to environment, and their influence on ecosystem processes, thus providing a mechanistic tool to monitor ecosystems' response to climate. The main aim of this thesis was to model the response of Mediterranean dryland ecosystems to climate, based on plant functional traits (PFT), using a spatial climatic gradient to predict changes over time. It aimed at selecting a trait-based indicator to track climate change effects on drylands, and contribute to improve land management and restoration strategies to mitigate land degradation. Although most trait-based metrics require the quantification of PFT in the field, there is no consensus about the best plant-sampling method to do it, to be used at a global scale. By comparing the performance of different methods, we demonstrated the advantages of the point-intercept method to perform fine-scale monitoring of PFT in drylands. Understanding which PFT respond to climate is essential to their use as indicators of ecosystems´ changes. Yet, this is not well established in drylands. We identified nine PFT responding to aridity. It affected PFT means, and reduced functional diversity. Studying climatic gradients in space to infer changes over time, requires a validation between the two. We found that inter-annual climatic fluctuations greatly affected PFT, indicating that functional changes are transitory. Yet, functional diversity was reduced under more limiting climatic conditions. Hence, we hypothesize that if drier conditions prevail in time, changes over time will approach those found along space. PFT are likely to respond in a more predictable way to environment than species diversity. We built a multi-trait functional diversity indicator, which showed a monotonic non-linear decrease with increasing aridity, responding in a more predictable way to climate than species diversity. Local biophysical factors modulate the effect of climate on plant communities. We explored the relative effect of these factors, and found that topo-edaphic factors played a major role shaping PFT associated to shrub encroachment. Climatic factors had a minor influence, suggesting that climate change will not promote shrub encroachment in Mediterranean drylands. PFT may be an important tool to improve dryland restoration. We provide a comprehensive overview of the current restoration practice in Mediterranean drylands, showing the need for a better integration of trait-based ecology into dryland restoration. By showing that PFT are consistent indicators of the impact of climate on dryland ecosystems, this work contributed to improve predictions on the effects of climate change on drylands, and enabled the development of a trait-based indicator which can be used to map areas at risk of desertification and land degradation, potentially at a global scale.

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Chapter 1

General Introduction

1 General Introduction

1.1 Climate change and ecosystem transitions

Climate change is already affecting natural and human systems on all continents, and its impacts will continue far beyond the 21st century (IPCC, 2014). Forecasted changes for global climate predict shifts in temperature and precipitation regimes and an increase in the frequency and intensity of climatic extremes (IPCC, 2014). The majority of the models addressing the effects of climate change on natural systems indicate alarming consequences for biodiversity (Bellard et al., 2012, Peñuelas et al., 2013). Biodiversity is the basis of ecosystem functioning (e.g. primary production, nutrient cycling, decomposition) and associated ecosystem services (e.g. fresh water, food, fuel, climate regulation) (Hooper et al., 2005). Hence, the impacts of climate change on biodiversity will be reflected in human livelihood and well-being (Mooney et al., 2009, Cardinale et al., 2012).

Changes in biodiversity as a consequence of climate change may be magnified by the synergistic interaction with, for instance, other global change drivers, or through positive biological feedbacks (Barnosky et al., 2012, Peñuelas et al., 2013). For example, rapid climate change combined with highly fragmented species ranges can be expected to enhance the potential for ecosystem collapse (Barnosky et al., 2012). This may increase changing rates and lead to the crossing of critical thresholds, causing ecosystem state transitions (Scheffer et al., 2001, Bestelmeyer et al., 2011, Barnosky et al., 2012). When ecosystems change to a diferent state, their capacity to provide ecosystem services changes as well (Carpenter et al., 2009). Sometimes, a small change in transition drivers may trigger large changes in ecosystems. These non-linear transitions are predicted to increase in general, as a response to global change drivers (Bestelmeyer et al., 2011), and may entail dramatic consequences for ecosystem services delivery, as has been reported for dryland agriculture, fisheries, and freshwater quality (MEA, 2005b). Previous studies reported non-linear transition in ecosystems caused by changes in climate (Scheffer et al., 2001). For instance, in the Western Antarctic Peninsula, an increase in annual mean air temperature of 2ºC led to the reduction of sea-ice duration, causing an abrupt shift in the breeding ranges of three closely related penguin species (Forcada et al., 2006, Bestelmeyer et al., 2011). Prior to the referred threshold, the abundance of those penguin species was was unresponsive to variation in sea-ice duration (Bestelmeyer et al., 2011).

Hence, to anticipate and adopt mitigation strategies to avoid unwanted ecosystem transitions due to climate change whenever possible, it is essential to detect early-warning signals of those shifts (Barnosky et al., 2012).

1.2 Biodiversity-based metrics to monitor ecosystem changes

The effect of environmental changes on ecosystems will vary according to their resilience, which depends on biodiversity. Therefore, to understand the effect of climate change on ecosystems we need measurable biodiversity metrics, which respond consistently to climate. Traditionally, ecologists have used the taxonomic diversity of communities to assess biodiversity changes in ecosystems (Pereira et al., 2013). The diversity of a regional pool of species may be divided into α and β diversity components: α corresponds to the diversity of spatially defined units, e.g. a community, while β measures differences in composition between communities (in space or time) (Magurran, 2013). Species richness has been the preferred metric to assess changes in communities' α diversity, induced by environmental changes or disturbance (Cadotte et al., 2011). However, environmental changes may lead to compositional shifts in communities over time (i.e., affecting β diversity), which may or may not precede species loss (Dornelas et al., 2014), but might nevertheless affect ecosystem functioning (Mouillot et al., 2011). For instance, a recent work reported a positive effect of plant species richness on several ecosystem functions in water-limited ecosystems (Maestre et al., 2012a). However, this effect was largely modulated by changes in community composition and functional characteristics (Maestre et al., 2012b). Species influence ecosystem processes via their functional traits (Hooper et al., 2005, de Bello et al., 2010, Mouillot et al., 2011). Functional traits are species attributes, measurable at the individual level, that influence their responses to environmental conditions (by affecting their fitness), or determine their influence on ecosystem properties (Lavorel and Garnier, 2002, Hooper et al., 2005) (Fig. 1.1). Species richness does not reflect species functional role in ecosystems, disregarding their functional uniqueness or redundancy within the community (Petchey and Gaston, 2006, Cadotte et al., 2011). Moreover, changes in functional traits in response to changing environments might be more consistent (i.e. show increase or decrease trends) than changes in species diversity, which may respond idiosyncratically or peak at intermediate levels of disturbance, potentially showing no signal of change (Mouillot et al., 2013). Functional traits can be studied in any type of community, independently of the species

identity, allowing the comparison of very distinct communities at a global scale. Therefore, functional traits provide a more universal and mechanistic understanding of species response to environment (Mason and de Bello, 2013), and may improve predictions of the effect of climate change on biodiversity and on ecosystem functioning (Suding et al., 2008).

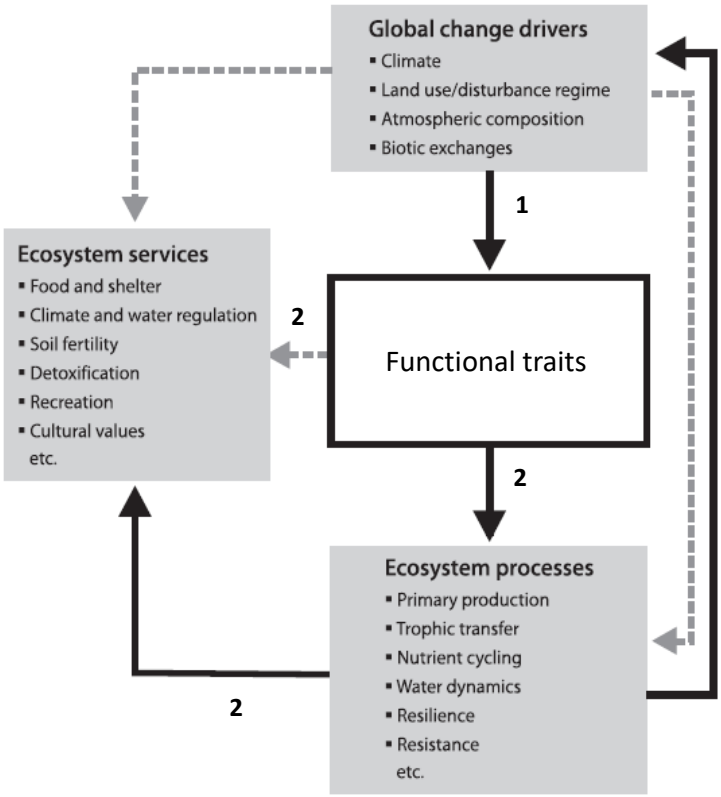

Figure 1.1. Functional traits: 1) influencing species response to environment and/or 2) affecting ecosystem processes and services. Adapted from Díaz et al. (2007a).

The functional traits of a biological community may be described by several trait-based metrics, comprising the mean of functional traits, often called functional structure, and their range or dissimilarity, i.e. functional diversity (Díaz et al., 2007b, Lavorel et al., 2008). The most used index to measure functional structure at the community-level is the community-weighted mean (CWM) (Garnier et al., 2007). It reflects the dominant traits in a community, and derives from the 'mass ratio hypothesis', according to which the effects of communities on ecosystem processes are largely determined by the traits of the dominant species (Grime, 1998). CWM enables to quantify community shifts in mean trait values due to environmental selection for certain traits, associated to changes in the abundance of dominant species.

Functional diversity may be divided into three components, namely functional richness, functional evenness, and functional dispersion, similarly to taxonomic diversity (Mason et al., 2005). However, functional dispersion, which considers trait abundance in addition to richness, has shown a better predictive ability than, for instance, functional richness (Schleuter et al., 2010, Mouillot et al., 2011). Functional diversity (FD) reflects the degree of functional dissimilarity within the community, and can be expressed through various indices (Mason et al., 2005, Villeger et al., 2008, Laliberté and Legendre, 2010). FD may be used to quantify the decrease or increase in trait dissimilarity along ecological gradients compared to a random expectation (i.e. trait convergence or divergence, respectively). Following the 'niche complementarity hypothesis', a higher FD is thought to reflect an increase in complementarity in resource use between species, and thus an increase in ecosystem functioning (Tilman et al., 1997). FD also allows the assessment of ecosystem resilience. The greater the presence of functionally similar species (higher redundancy), the higher the probability that disturbance-induced local extinctions of species will be compensated by the presence of similar species, ensuring higher ecosystem resilience (Pillar et al., 2013). With the aim of resuming the functional diversity of multiple traits within a single FD value, thereby allowing to estimate the 'functional trait space' occupied by a community, several multi-trait indices have been developed over the last decade (Villeger et al., 2008, Laliberté and Legendre, 2010, Schleuter et al., 2010). However, the integration of multiple traits into one index has to take into account single-trait trends and their possible co-variation, to avoid its misinterpretation (Butterfield and Suding, 2013). Both metrics (CWM and FD) were reported to respond to major environmental filters, disturbance regime or biotic interactions, and to affect major ecosystem processes like primary production or decomposition rates (de Bello et al., 2010, Mouillot et al., 2011, Mouillot et al., 2013, Valencia et al., 2015). Therefore, trait-based metrics might be a good approach to assess climate change impacts on ecosystems.

1.3 Drylands and climate change: desertification and land degradation

Dryland ecosystems are highly vulnerable to climate change (Sala et al., 2000, Reynolds et al., 2007a). They are characterized by low precipitation amounts which do not compensate for the evaporative demands imposed by high temperatures and solar radiation, thereby exhibiting high aridity levels (Reynolds et al., 2007a, MEA, 2005a).

Hence, climate change, particularly the expected increase in temperature and the predicted changes in precipitation patterns, are likely to further aggravate water-limitations in these ecosystems (Maestre et al., 2012c). Drylands occupy 41% of the land surface and are inhabited by more than 38% of the human population (Reynolds et al., 2007a). They include dry sub-humid, semi-arid, and arid areas classified according to the aridity index of United Nations Environment Programme (UNEP), representing the ratio of mean annual precipitation to annual potential evapotranspiration (Middleton and Thomas, 1992) (Fig. 1.2).

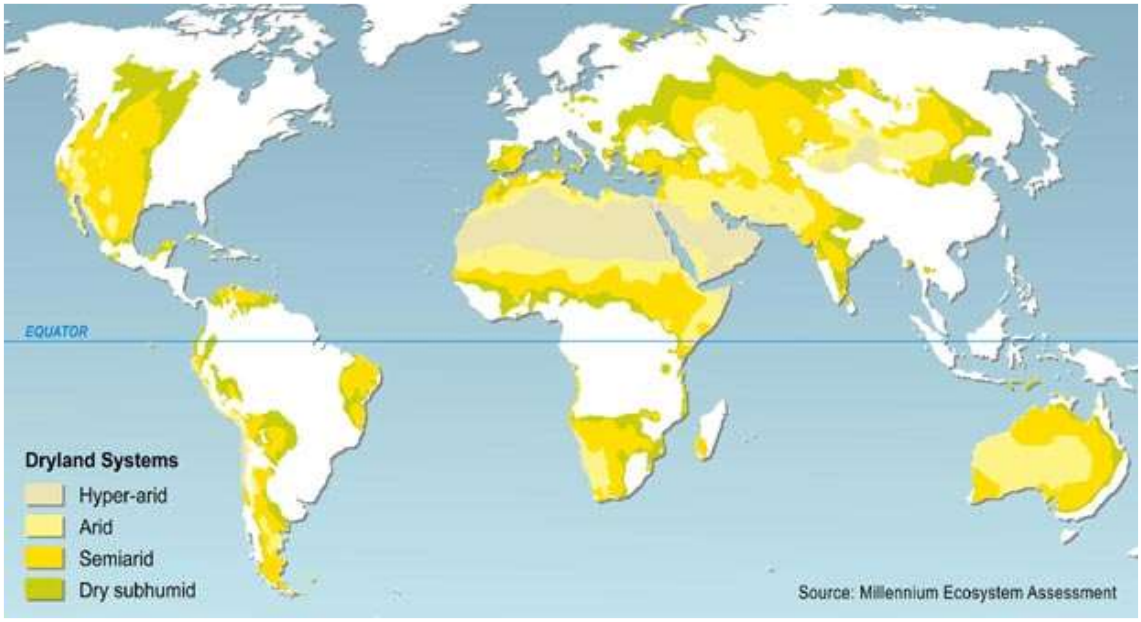

Figure 1.2. World distribution of drylands and their subtypes (Aridity index range: 0.05–0.65). Adapted from Millennium Ecosystem Assessment (2005b).

Climatic constraints limit the productivity of drylands, increasing their susceptibility to wind and water erosion. These climatic limitations, coupled with intense human activity (e.g. agriculture, grazing, and deforestation) lead to desertification and land degradation. Desertification is defined by the United Nations Convention to Combat Desertification as land degradation in drylands, i.e. the reduction or loss of the biological or economic productivity of drylands, resulting from various factors, including climatic variations and human activities (UNCCD, 2012). Land degradation already affects 10–20% of drylands worldwide, and its extent and intensity are expected to increase substantially as a result of climate change, population growth, and land cover changes (IPCC, 2014, MEA 2005c).

In drylands, precipitation exhibits a highly variable distribution in space and over time, due to seasonal and inter-annual climatic fluctuations (Noy-Meir, 1973). Since precipitation amounts are low, local soil characteristics (e.g., soil water holding capacity) and topography (e.g. aspect, slope) largely determine how much water is available to plants, influencing vegetation regeneration, structure and cover (Noy-Meir, 1973, Gómez-Plaza et al., 2001, Príncipe et al., 2014). As a consequence, plant spatial distribution is generally patchy, with discontinuities in biomass production, which affect soil fertility, soil microbiota and rate of decomposition (Schlesinger and Pilmanis, 1998, Aguiar and Sala, 1999, Maestre and Cortina, 2002).

Despite their aridity, and also as a consequence of high spatial and temporal heterogeneity (Chesson et al., 2004), drylands support a rich diversity of plant and animal species (Davies et al., 2012), that have evolved and adapted to cope with water and nutrient limitations. The most representative types of land-cover are shrubland (24% of drylands), cropland (20%), savanna (15%), and grassland (13%) (Reynolds et al., 2007b). Plant species exhibit a variety of mechanisms to avoid (e.g. annual life-cycle) or to tolerate drought (e.g. woody perennials), and to deal with disturbance pressures such as fire and herbivory (Noy-Meir, 1973, Davies et al., 2012). Drylands' biodiversity interacts with abiotic factors to determine ecosystem functioning (e.g., productivity, nutrient fluxes) and resilience (i.e., the ability to return to a previous state after disturbance), both of which are critical to ensure the provision of ecosystem services (MEA, 2005a). From less to more arid areas, primary productivity decreases (MEA, 2005a, Davies et al., 2012). As climate change predictions point to an overall increase in aridity in drylands (Dai, 2013, IPCC, 2014), it is expected to further reduce productivity over time. However, the effects of increasing aridity on dryland ecosystems' biodiversity and functioning, and on associated ecosystems services, remain unclear.

1.4 Plant functional traits response to climate

It has been suggested that higher aridity may lead to lower species richness, because primary productivity falls and reduces the availability of resources for wildlife (Davies et al., 2012). However, this hypothesis lacks further scientific evidence. High spatial and temporal heterogeneity found in drylands may also provide a higher niche differentiation, and thus favor the co-existence of more species with different requirements. Indeed, many dryland areas are considered biodiversity hotspots (Myers et al., 2000).

Moreover, information only about species richness does not provide insights about the effects of increasing aridity on ecosystem functioning (Cadotte et al., 2011). As functional traits are the means by which species influence ecosystem processes, a functional approach in addition to a taxonomic one may be more revealing of the effect of aridity on plant communities and its consequences for ecosystem processes. It may enable the detection of critical abundance changes (e.g. of a set of traits under pressure) able to affect ecosystem functioning, being reactive even when species richness is not (Mouillot et al., 2013). The diversity of functional traits (FD) is closely related to ecosystem functioning (Mouillot et al., 2011) and resilience (Volaire et al., 2014). As such, we would expect changes in plant communities' FD to be more revealing of ecosystem functioning loss due to desertification and land degradation in drylands, than changes in taxonomic diversity. To confirm this hypothesis, it is of paramount importance to understand if FD responds to climatic variation in a consistent way. If this is the case, functional trait metrics could be used as indicators to track the effect of climate change on dryland ecosystems. The development of indicators able to unveil the impacts of climate change on dryland ecosystems to be used at global scale is much needed, and such indicators have long been requested, for instance, by the United Nations Convention to Combat Desertification (UNCCD), as well as by the UN Conventions on Biological Diversity (UNCBD), and on Climate Change (UNCCC). The development of a trait-based indicator would require the study of functional traits of plant communities along high-resolution climatic gradients, to allow the modelling of the response of FD to climatic variation.

Aridity may affect the relative abundance of plant traits associated to stress-tolerant versus stress-avoidant strategies (Ackerly et al., 2002, Gross et al., 2013), and growth-forms (Fay et al., 2002). It may affect plant height (Gross et al., 2013), flowering timings (Hänel and Tielbörger, 2015, Kigel et al., 2011), and plant reproductive strategies (Baker, 1972, Arroyo et al., 2006, Volis and Bohrer, 2013, Gremer and Venable, 2014). Aridity might also select species with similar trait values within communities (lower functional diversity), by reducing the abundance or filtering out of the community less 'adapted' species (Grime and Díaz, 2006, Cornwell and Ackerly, 2009). Regardless of previous studies involving plant functional traits and aridity, there is no clear picture about which are the main plant traits responding to aridity, at the whole-community level. This is because previous studies relied largely on low-resolution gradients (De Bello et al., 2005, Lavorel et al., 2011, Frenette-Dussault et al., 2012), focused mostly on a single trait-based metric (e.g. CWM) (Barboni et al., 2004), and were based primarily on perennial species (Gross et al., 2013, Valencia et al., 2015, Costa-Saura et al., 2016, Bagousse-Pinguet et al., 2017). The latter reason is

a major shortcoming, considering that annual species constitute an important part of the diversity of dryland plant communities, often bearing most of the floristic diversity (Noy-Meir, 1973, Aronson et al., 1993). In addition, annuals play a critical role in ecosystem functioning, e.g. in energy flow and nutrient cycling (Baldocchi et al., 2004, Gilliam, 2007, Ramos et al., 2015), and may respond more readily than perennials to environmental changes due to their faster turnover. Annual species are also quite relevant for ecosystem services provision in drylands, as they are frequently the main food source for livestock, the primary use of drylands (Asner et al., 2004). The response of plant traits to aridity quantifying different functional trait metrics at the whole-community level (considering also annual species) is not well established in drylands. For many plant traits it remains unclear whether their mean (CWM) and range (FD) respond to aridity, and how (i.e. if they increase or decrease). Clarification could be improved if works contemplated a higher number of plant traits addressed at the whole-community level along climatic gradients with higher resolution.

The response of trait-based metrics to environmental change, similarly to taxonomic diversity, may depend on the spatial scale of analysis (local versus regional). A recent work in plant communities of the French Alps found hierarchical effects of environmental gradients on functional trait metrics: large-scale environmental factors (e.g. temperature) were found to predominantly shape CWM, while fine-scale factors (topography and soil characteristics) mostly influenced FD (Bello et al., 2013). However, this may not always be the case, depending on the type of community and on the environmental driver studied. Trait mean (CWM) may also change considerably along finer-scale local gradients, e.g. of land use intensity (Lavorel et al., 2011), or grazing (McIntyre and Lavorel, 2001). Conversely, FD can change along large-scale climatic gradients as well (Valencia et al., 2015, Bagousse-Pinguet et al., 2017), e.g. as a result of species/traits environmental filtering from an available regional pool. This highlights the importance of considering different (complementary) functional metrics to improve predictions of ecosystems' response to environmental change, as different metrics may respond differently (Bello et al. 2013).

1.5 Standard method to sample traits in dryland plant communities

To measure functional-trait metrics in plant communities and to obtain comparable estimates along space and time, we need a 'standardized' field sampling method that can be used by different observers across different regions. Standardized methodologies to measure plant traits value *per se* are quite well established (Pérez-Harguindeguy et al., 2013). This also contributed to build regional and global plant trait databases (Kattge et al., 2011), on which many trait-based studies rely to retrieve species trait values (Díaz et al., 2016, Kunstler et al., 2016). However, there is no consensus on the best sampling method to assess species/traits relative abundances in the field, which are required to compute the most informative functional-trait metrics (Schleuter et al., 2010, Mouillot et al., 2011). Plant biomass (Prieur-Richard et al., 2002), frequency (De Bello et al., 2005), and, most commonly, cover (Lavorel et al., 2008, Frenette-Dussault et al., 2012) are among the plant abundance measures used to estimate functional-trait metrics. Cover estimates obtained by different methods vary considerably (Abrahamson et al., 2011) and may therefore affect estimates of functional-trait metrics. Differences in these methods can affect, for instance, the 'amount of trait diversity' analysed (Májeková et al., 2016), i.e. the number of species included in the 80% 'dominance' threshold of the relative cover, considered an adequate proportion to functionally characterize a plant community (Garnier et al., 2004, Pakeman and Quested, 2007). Although considerable attention has been devoted to comparisons of plant-sampling methods over the last 20 years (Floyd and Anderson, 1987), few studies have assessed the performance of different cover-sampling methods to estimate functional-trait metrics (Abrahamson et al., 2011). Therefore, the definition of an adequate and reproducible sampling method for the non-destructive fine-scale monitoring of functional-trait metrics in drylands, is essential to allow monitoring over space and time by different observers and across different ecosystems.

1.6 Space for time substitution

The impacts of climatic variation on biodiversity are often studied assessing changes along spatial climatic gradients to infer changes over time, using the so-called 'space-for-time substitution', due to the general lack of information over time (Blois et al., 2013). This method assumes that ecosystems will respond to changing climate over time in the same

way that they now vary over space (Dunne et al., 2004). However, this assumption can be misleading in cases where historical attributes of sites have particular effects on ecosystem structure and function (Vitousek, 1994, Dunne et al., 2004). For instance, long-term adaptation to local climatic conditions, or fine-scale environmental heterogeneity, may confound the straightforward use of spatial gradients to predict responses to climate over time (Dunne et al., 2004). A possible way to overcome this limitation is to integrate the two approaches, i.e. assessing spatial and temporal trends within a single study, thereby enabling to distinguish among consistent, transient, and context-dependent ecosystem responses to climate (Dunne et al., 2004). It provides a way to validate the 'space-for-time substitution' method, by assessing if what happens along space, holds in time. This validation is particularly relevant in systems subject to high inter-annual climatic variability, such as drylands. Climatic fluctuations between years have important impacts on plant productivity (Yang et al., 2008, Miranda et al., 2009, Sala et al., 2012), and are known to affect the composition and diversity of plant communities (Peco et al., 1998, Miranda et al., 2009) and their functional characteristics (Pérez-Camacho et al., 2012, Carmona et al., 2015), particularly in communities dominated by annual species. However, few studies compare the outcomes of spatial gradients with vegetation changes over time, (but see Aronson and Shmida, 1992, Sala et al., 2012, Cleland et al., 2013). A recent work comparing changes in plant productivity along space and time in a wide variety of drylands from different continents, found a much weaker temporal relationship between precipitation and productivity at a given site, than in space, built across many sites (Sala et al., 2012). The authors suggest that the temporal models describe the transient response of the plant communities to climate change and that the spatial model describes the equilibrium condition to which temporal trends converge, when 'slower ecosystem variables' such as the relative species abundance start changing, if environmental conditions persist (Sala et al., 2012). In other words, they suggest that temporal trends will resemble predictions of the spatial model when the ecosystem structure reaches equilibrium, i.e. when enough time passes under consistent environmental conditions in the long run (Sala et al., 2012). Hence, understanding ecosystem short-term and long-term responses to climate, and how they interact with local factors, is essential to predict climate change impacts on the functional structure and diversity of dryland plant communities.

1.7 The role of local drivers and management

Climate has a major influence on the structure and diversity of plant communities, acting at global to regional spatial scales. However, the effect of climate on plant communities is also largely modulated by local factors, both biophysical (e.g. soil characteristics, topography) and anthropogenic (e.g. grazing). Topo-edaphic factors largely control water availability to plants, particularly in water-limited ecosystems (Gómez-Plaza et al., 2001, Colgan et al., 2012, Sala and Maestre, 2014). These factors, in interaction with the amount and timing of precipitation (e.g. if it occurs during warmer or colder periods), determine how much moisture is available to plants at different soil depths, and for how long, thus affecting vegetation patterns (Sala et al., 1997, McAuliffe, 2003). In addition, the effect of regional and local biophysical factors will depend also on the local historical land use and management, making the disentangling of such interactions a challenging task. The overwhelming majority of drylands serve as rangelands (>75%), grazed by domestic livestock or wild animals, while ca. 20% are rainfed or irrigated cropland (Reynolds et al., 2007b). Hence, human activities such as agriculture, livestock management, shrub clearing, or deforestation also affect plant cover and vegetation structure (Reynolds et al., 2007b). Shrub encroachment, defined as the increase in density, cover and biomass of native woody species (Van Auken, 2009), has been reported in many drylands worldwide (Roques et al., 2001, Maestre et al., 2009, Van Auken, 2009, Caldeira et al., 2015, Eldridge and Soliveres, 2015), as the result of both natural and human-induced processes. It has been described as a self-reinforcing and often abrupt shift in space and time from grasslands into shrub-dominated communities, sustained by a positive feedback between vegetation and environmental conditions (D'odorico et al., 2012). Some authors see it as a land degradation process (Schlesinger et al., 1990, MEA, 2005c), frequently from the point of view of livestock production, which is a primary use of drylands. Other authors argue it may improve some aspects of ecosystem functioning (Maestre et al., 2009, Daryanto et al., 2013, Gómez-Rey et al., 2013) (Fig.1.3).

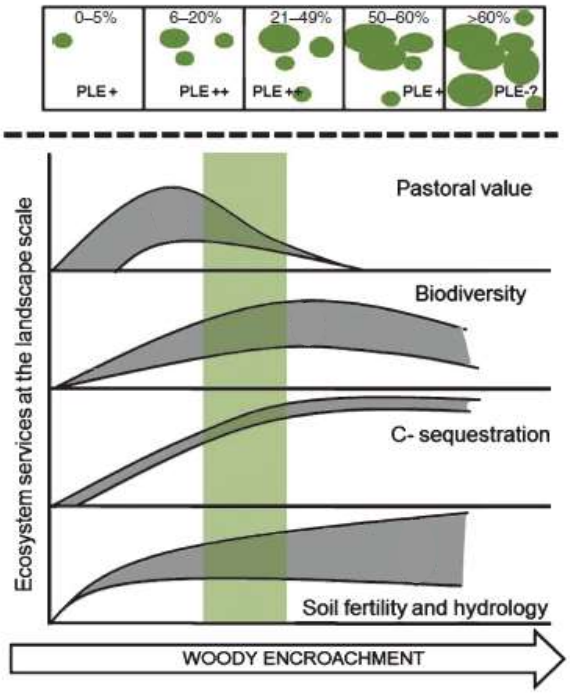

Figure 1.3. Upper panel: the effects of individual shrub patches (patch-level effect; PLE) on ecosystem services across Australian woodlands are predicted to remain positive until woody cover reaches about 60%; Lower panel: the predicted responses of five ecosystem services to increasing shrub encroachment, with the range of values shown in the shaded envelope. The vertical green stripe indicates the range in woody cover values that maximize the level of all ecosystem services simultaneously. Adapted from Eldridge and Soliveres (2015).

Previous works identified a combination of multiple factors responsible for shrub encroachment, including human activities (e.g. grazing intensity, shrub clearing), fire frequency and climate change (Eldridge et al., 2011). Most studies addressing the causes of shrub encroachment are from North American grasslands (Van Auken, 2009), African savannas (Roques et al., 2001), or Australian woodlands (Eldridge and Soliveres, 2015). However, the relative effects of climate and of local factors (e.g. soil, topography) on shrub encroachment and on associated plant functional changes are clearly lacking evidence from Mediterranean Basin drylands. Moreover, traditional management strategies to deal with shrub encroachment, relying mainly on mechanical shrub clearing, do not have in most cases sustainable positive effects on forage production (Rango et al., 2005, Eldridge and Soliveres, 2015). Considering that shrub encroachment has important consequences for dryland ecosystem services, and that considerable resources are allocated to unsuccessful shrub removal programs, further evidence is needed to help improve management actions aimed at reversing it and to anticipate potential impacts of climate change on Mediterranean Basin ecosystems.

1.8 Plant functional traits as tools for dryland restoration

In drylands, human activities coupled with climatic constraints, led to extensive areas of degraded lands exhibiting low biological productivity and slow ecosystem recovery rates after disturbances or abandonment of land use (Le Houerou, 2000). High water stress together with more intense and/or frequent disturbances often reduce ecosystem resilience, generating a positive feedback which exacerbates land degradation. Land degradation reduces the ability of ecosystems to deliver ecosystem services, compromising people's livelihood and well-being (MEA, 2005c, Reynolds et al., 2007b). Dryland degradation may be further aggravated by climate change that is expected to generate warmer and drier conditions and a higher frequency of extreme events, i.e., heat waves, droughts and floods (MEA, 2005c, IPCC, 2014).

In severely degraded areas with low resilience, restoration is the main means to reverse land degradation, and to restore ecosystems' composition, functioning and sustainability, thus contributing to improve the welfare of local populations (Zucca et al., 2013, Suding and Higgs, 2015). Restoration actions are particularly challenging under the stressful conditions found in drylands (Vallejo, 2009). During the first half of the 20th century, many large restoration projects were conducted in drylands (Birch et al., 2010, Bainbridge, 2012), but many flawed and inappropriate approaches were used. This happened for several reasons, including the limited understanding of dryland ecosystems, ignorance of past restoration experiences in drylands, and the use of models from humid areas by most researchers and land managers (Bainbridge, 2012). In addition, in many cases, e.g. across the Mediterranean Basin, restoration efforts relied on the introduction of a few fast-growing tree species, and intended to combine forest productivity with hydrological watershed protection (Vallejo, 2009). Despite their contribution to reduce erosion and increase plant cover, in many cases they led to systems with low productivity and diversity, failing to improve ecosystem functioning (Goberna et al., 2007, Cortina et al., 2011). Fostered by criticism and also by legislation initiatives and environmental policies, this earlier restoration paradigm has been progressively replaced by a more ecosystem-based approach, with the introduction of more plant species, and focused also on the functioning of the restored ecosystems (Vallejo, 2009, Cortina et al., 2011). More recently, functional ecology theories and research began to be progressively integrated into restoration science and practice (Brancalion and Holl, 2015, Kunstler et al., 2016). Selecting species with particular functional traits to be used in restoration can be critical to overcome common early limitations to ecosystems' recovery in dryland areas, and thus to restoration success

(Vallejo et al., 2012). Plant traits may be an effective 'tool' to reduce erosion (e.g. root architecture), improve soil quality and nutrient cycling (e.g. nitrogen-fixing ability, leaf decomposability), influence species facilitative/competitive interactions (e.g. height, growth-form) or biotic fluxes (e.g. dispersal strategy, palatability, type of fruit), and thus enhance ecosystem functioning and accelerate its recovery (Suding et al., 2008). In addition, plant functional traits can be useful indicators of the recovery of ecosystem functioning (Hooper et al., 2005) and associated ecosystem services (Díaz et al., 2007c, de Bello et al., 2010) following restoration actions. Plant traits related to drought and fire-tolerance, for instance, may be critical for drought adaptation and resilience of the restored ecosystem, particularly under a climate change scenario (Sterk et al., 2013, Suding et al., 2015). However, to what extent research on plant functional traits is being considered and integrated into drylands' restoration practice remains unclear. A comprehensive overview of the current restoration practice implemented in drylands is needed in order to critically analyse whether trait-based ecology theories and research are being incorporated into restoration practice.

1.9 Objectives and structure of the thesis

The main aim of this thesis was to model the response of Mediterranean dryland ecosystems to climate based on plant functional traits, using a spatial climatic gradient, to predict changes over time due to climate change. While contributing to increase scientific knowledge on dryland ecology, this work aimed to: select a trait-based indicator to track climate change effects on dryland ecosystems; contribute to the improvement of land management strategies to mitigate desertification and land degradation; improve the available restoration tools to recover ecosystem functioning in degraded drylands. The development of indicators to track climate change impacts on dryland ecosystems will fulfill the need expressed by the United Nations Convention to Combat Desertification (UNCCD), as well as by the UN Conventions on Biological Diversity (UNCBD), and on Climate Change (UNCCC), for biodiversity monitoring indicators to be used at a global scale.

The rationale of the work consists in understanding climate-driven desertification and land degradation processes in drylands through plant functional traits, and conversely, contribute to the integration of that information to support dryland restoration (Fig. 1.4).

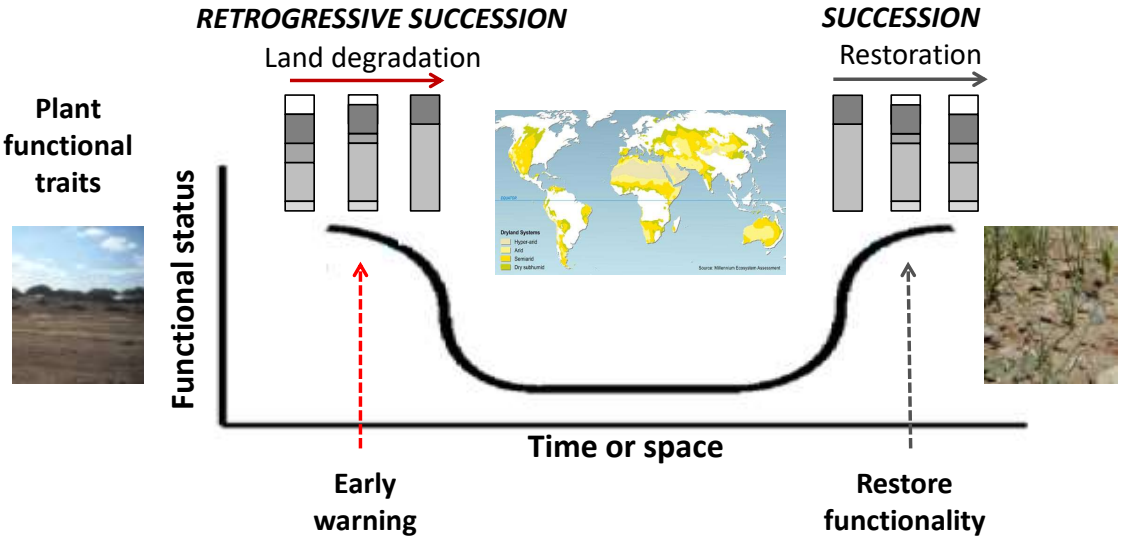

Figure 1.4. Conceptual scheme of the rationale of the present work.

Chapter one provides a general introduction of the importance of predicting climate change impacts on biodiversity, highlighting the potential and advantages of using functional traits to monitor changes at the ecosystem level. The specific case of drylands is presented, as being particularly susceptible to climate change. It is expected to enhance ongoing desertification and land degradation in drylands, compromising ecosystems' capacity to deliver ecosystem services. This will frame the need to model the response of dryland ecosystems to climate in space, allowing the prediction of their response over time, using plant functional traits. This information could be used to develop trait-based indicators capable of tracking the effect of climate on dryland ecosystems, and to improve land management strategies and restoration tools to mitigate dryland ecosystems' degradation.

Most trait-based metrics require the quantification of species and/or traits in the field, in order to obtain community weighted values. Although the quantification method used is known to influence trait metrics estimates, a consensus has not been reached on the best plant-sampling method to detect spatial and temporal changes in functional traits in dryland communities. Moreover, there is a growing demand for reproducible methods that can be used at a global scale and by different observers. In **Chapter two** we compare the performance of different field sampling methods to estimate trait-based metrics, and indicate the most appropriate to perform non-destructive fine-scale monitoring of functional structure and diversity in semi-arid areas.

To model the response of dryland ecosystems to climate, we proposed to study changes in functional traits metrics along a spatial climatic gradient, to predict changes over time due to climate change, using a 'space for time substitution'. The first step, should be the selection of traits which are responsive to climate and related to specific ecosystem functions. Aridity provides an integrative measure of the predicted changes in precipitation and temperature in drylands. Therefore, aridity was used to define a climatic gradient. Previous studies related plant functional traits and aridity, however mostly focusing on a single functional trait metric and primarily on perennial species. Hence, there is no clear picture about which are the main plant traits responding to aridity at the whole-community level, i.e, comprising annual and perennial species. In **Chapter three,** we study how several functional traits related to plant establishment, growth, reproduction, dispersal and persistence, change along a high-resolution spatial aridity gradient. Plant functional traits (of annual and perennial species) responding to aridity were identified, and changes in their mean and range were quantified, thus assessing two essential and complementary facets of functional trait variation.

A 'space-for-time substitution' approach was used to study aridity impacts on plant communities' functional metrics in the previous chapter. The validation of this approach, requires an understanding of how changes found along space, match those occurring over time. In addition, dryland ecosystems are subject to high inter-annual climatic variations, which may affect functional trait metrics. In **Chapter four,** the effect of inter-annual climatic fluctuations on plant community functional traits was evaluated, as well as the way these fluctuations interact with long-term climate and local topography. Nine sampling sites located along a spatial climatic gradient were selected, and studied in four climatically contrasting years. It was possible to compare changes in space and time and evaluate the predictive power of different environmental drivers to explain changes in the mean and range of functional traits.

Functional trait metrics provide an alternative to the use of taxonomic diversity metrics as ecological indicators of environmental changes, which respond rapidly and consistently across taxa and ecosystems types to multiple disturbances, being a potentially better indicator of environmental change effects on ecosystems. This is the reason for the inclusion of functional traits in the list of essential biodiversity variables to be used in monitoring programs worldwide. In **Chapter five,** the response of several traits was integrated in a multi-trait functional diversity index, to be used as an indicator of dryland

ecosystems' response to climate. Contrary to species diversity, this multi-trait index showed a monotonic non-linear decrease with increasing aridity, proving to be a better indicator.

Other local factors might confound the response of plant functional traits to climate, particularly in water-limited ecosystems. Local topography and soil characteristics modulate the effect of precipitation on plant communities, by determining how much moisture is available to plants at different soil depths, and for how long. Therefore, understanding how climatic factors acting at a regional scale interact with local environmental conditions is essential to model the response of dryland plant communities and associated ecosystem services to climate change. In **Chapter six** the relative effects of climatic, topographic and edaphic factors on trait-based metrics of plant communities, and particularly on shrub encroachment, are explored. A model to predict shrub encroachment at a local scale, now and under a climate change scenario is presented, to help define more cost-effective and sustainable management strategies to deal with shrub encroachment in Mediterranean ecosystems.

The integration of trait-based ecology into restoration practice may help improve restoration efforts in degraded drylands. However, to what extent research on plant functional traits is being considered and integrated into drylands' restoration practice remains unclear. In **Chapter seven** the responses to an on-line survey addressed to dryland restoration practitioners are analysed, and a comprehensive overview of the current restoration practice in Mediterranean drylands is presented. Indications are provided on what is needed to improve and promote ecological restoration in drylands, including the need to foster the use of trait-based indicators (i.e. the presence and abundance of critical functional traits) in restoration monitoring and evaluation.

Chapter eight summarizes the major findings of this work for the understanding of the response of plant functional traits to climate-driven desertification and land degradation in drylands. The response of plant functional traits to climate is discussed, namely the predictability of their responses, and their global application in other dryland ecosystems, e.g. in North and South America. Afterwards, we discuss the contribution of our findings to improve management and restoration efforts to mitigate desertification and land degradation in these ecosystems. By integrating the knowledge gathered over the previous chapters, this chapter also opens new perspectives on the potential of plant functional traits as universal indicators to monitor the response of dryland ecosystems to changing climate, and identifies future challenges and research lines that can be drawn from this work.

1.10 Study area

The studies described along the thesis were conducted in southwestern Iberian Peninsula, Portugal. Climate is Mediterranean, with precipitation occurring mainly between autumn and spring, and hot and dry summers. Mean annual precipitation (50 yrs. average) along the study area varied from 520 mm to 634 mm, with a high inter-annual variability (annual precipitation can vary by more than 100 mm between years), and mean annual temperature was ca. 16.3 ºC. The study area comprises dry sub-humid and semi-arid climates. The landscape is characterized by moderate slopes (from 0.8 to 25.5º) and dominated by savanna-like Holm-oak woodlands (*Quercus ilex* L. subsp. *rotundifolia*). Holm-oak woodlands are the dominant cover type in the drier areas of the Iberian Peninsula, mostly under dry sub-humid and semi-arid climates (Olea and San Miguel-Ayanz, 2006, Belo et al., 2009). In the more coastal areas, where precipitation levels are slightly higher (>600 mm), Cork-oak (*Quercus suber* L.) tends to dominate (Surova and Pinto-Correia, 2008) (Fig 1.5a). These ecosystems are called 'montado' in Portugal and 'dehesa' in Spain. They consist of semi-natural multifunctional systems with a traditional low intensity agro-silvopastoral use (e.g. extensive grazing, occasional seeding of pastures and shrub clearing), and are quite important in the economy of rural areas (Barbero et al., 1992, Surova and Pinto-Correia, 2008, Belo et al., 2009).

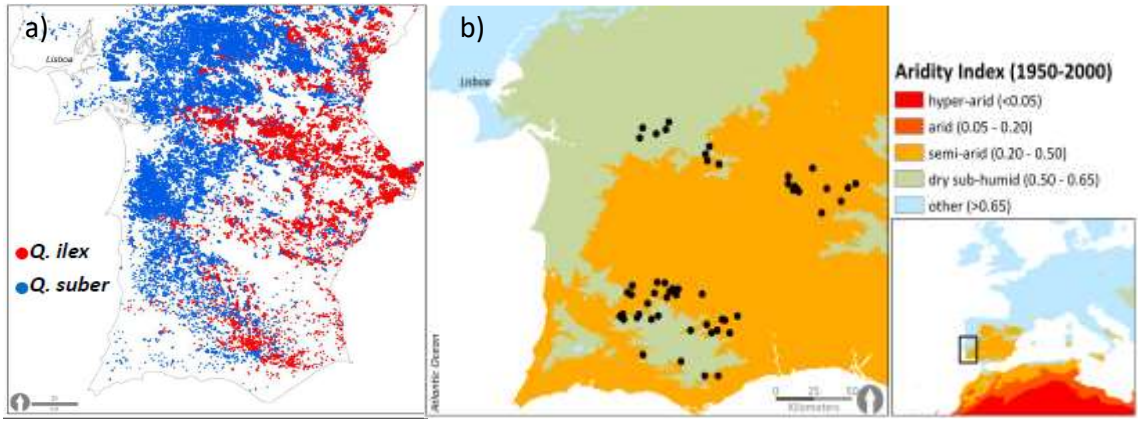

Figure 1.5. Maps of the study area: a) distribution of Holm-oak (*Quercus ilex* L. subsp. *rotundifolia*) and Cork-oak (*Quercus suber* L.) in the south of mainland Portugal. The map was built using the National Forest Inventory (ICNF, 2006) classifying each point according to the dominant species; b) sampling sites distribution (black dots) along dry sub-humid and semi-arid areas.

Holm-oak woodland are heterogeneous landscapes which support a remarkably rich biodiversity (Pereira and Da Fonseca, 2003). They are composed of a sparse tree cover (<

40 trees per ha, on average) (Amaral et al., 1997) and an understory of semi-natural grasslands dominated by annual species, intermingled with shrubland patches (Barbero et al., 1992) (Fig. 1.6). Although the plant community is generally well adapted to drought, and resilient to seasonal and inter-annual variations in precipitation, high aridity levels coupled with human activities, increase the susceptibility of these ecosystems to desertification and land degradation (Belo et al., 2009). In recent decades, a climatic trend of increasing aridity has been observed in the south of Portugal, in accordance with climate change predictions of increased aridity in the near future (Costa et al., 2008). Therefore, climate change may lead to further land degradation, compromising the provision of ecosystem services to local population (Belo et al., 2009).

The field work developed in the thesis was carried out along a regional climatic gradient, based on aridity index values (the ratio of mean annual precipitation to annual potential evapotranspiration) for the period 1950–2000 (Middleton and Thomas, 1992, Trabucco and Zomer, 2009), which ranged from 0.42 to 0.56 (Fig 1.5b). In order to reduce confounding effects of other factors and ensure homogenization, a pre-selection of sites was made prior to the stratification based on aridity. The pre-selected sites had moderate to low grazing intensity, no agricultural activity in recent years, altitude between 150 and 300 m, soil dominantly acidic (pH<6.5) laying on sedimentary and metamorphic rocks, and no fire records. Low grazing intensity and the absence of recent agricultural activities were empirically confirmed in the field by inspecting evidence of grazing in vegetation, the amount of ungulate pellets, and absence of recent soil tillage.

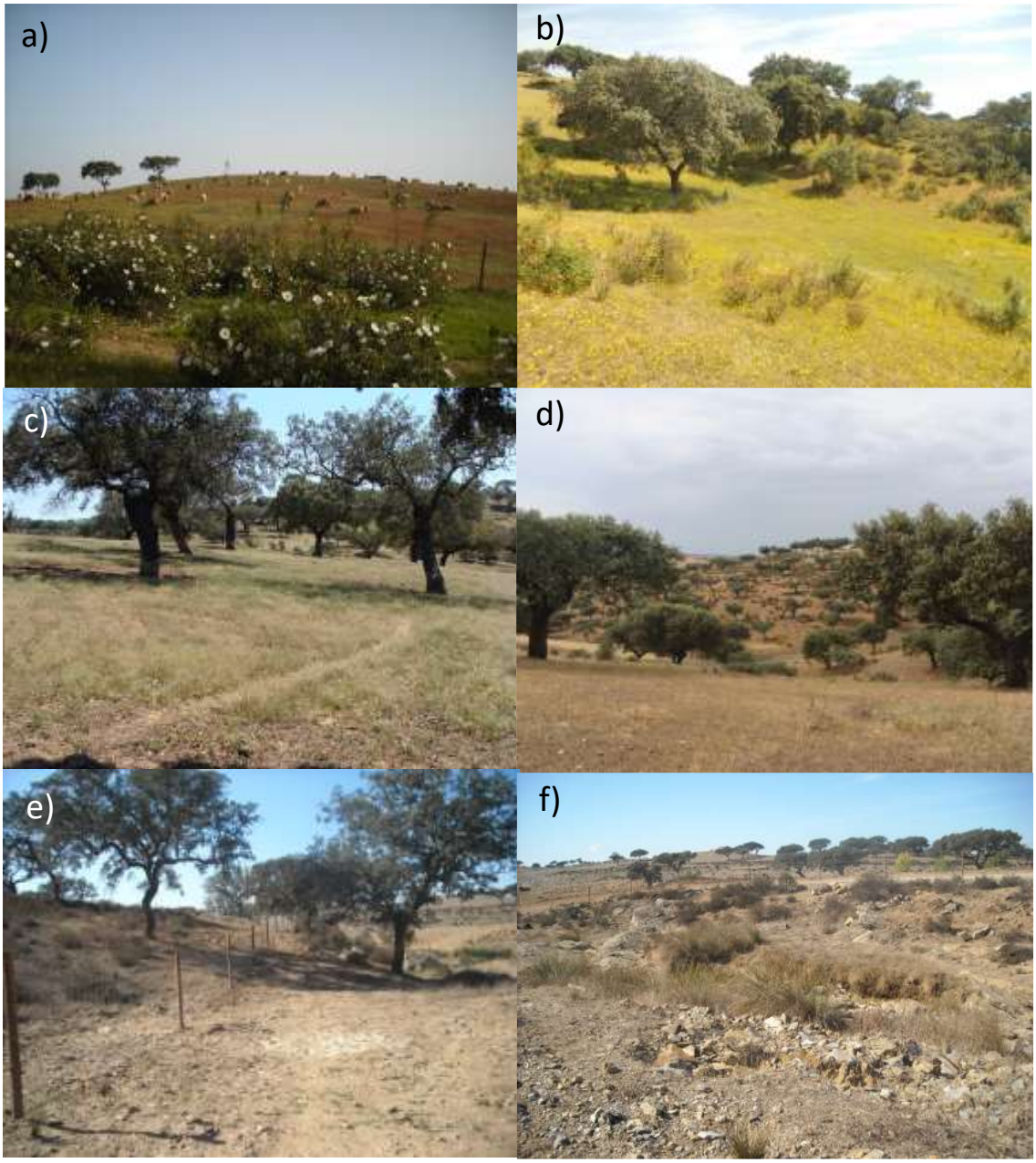

Figure 1.6. General overview of different landscape perspectives of Holm-oak woodlands in the south of Portugal: a, b) during spring; c) late spring; d, e, f) during summer.

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Chapter 2

Advantages of the point-intercept method for assessing functional diversity in semi-arid areas

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2 Advantages of the point-intercept method for assessing functional diversity in semi-arid areas

2.1 Abstract

Semi-arid areas are particularly susceptible to the loss of biodiversity as a consequence of global change. Species functional traits are key drivers of functioning and resilience of ecosystems, thus monitoring of functional trait diversity is urgently needed. The assessment of functional diversity requires the quantification of species and/or their traits in the field, though there is no consensus on the best plant-sampling method to be used. The aim of this study was to compare the performance of the point-intercept (PT) method with two area-based approaches, the modified-Whittaker (MW) and Dengler (DE) methods, to assess functional diversity in semi-arid areas. The herbaceous community of a savanna-like Mediterranean woodland was surveyed at the two extremes of a regional precipitation gradient (dry to wet). Efficiency in the quantification of species/traits, precision of cover estimates, and their effect on functional diversity metrics computed for eight functional traits were compared. Results showed that the examined methods differed in their efficiency in quantifying species/traits in both sites. With the DE method, fewer species were detected than with the MW and PT methods, which yielded similar values. The PT method had a higher precision in the quantification of both dominant and non-dominant species/traits. It also had a higher community evenness, mainly in the wet location, which allowed the analysis of a greater number of species/traits within the 80% “dominance” threshold (i.e., species representing 80% of the relative cover of community), a critical aspect of functional diversity assessments. In addition, the PT method yielded higher estimates for multi-trait functional evenness, as well as different estimates (either higher or lower than MW and DE) of single-trait community weighted means (for N-fixing ability and flowering onset), functional dispersion (for N-fixing ability and specific leaf area), and functional evenness (for height and flowering onset). In spite of the observed differences among methods in the assessment of functional diversity, the PT approach demonstrated important advantages in the non-destructive, fine-scale monitoring of semi-arid areas, where “less dominant” species may play a critical role.

Keywords: Dengler method; drylands; field plant sampling; functional structure; functional diversity; grassland; Modified-Whittaker; point-intercept method

2.2 Introduction

Semi-arid ecosystems are characterized by water scarcity and often by low soil productivity. Due to global change, they are highly vulnerable to losses of biodiversity, which underpins many critical ecosystem services (Reynolds et al. 2007). The more dramatic effects on these ecosystems are often preceded by subtle changes in relative species' abundance and/or in the dominance of specific functional traits (Chapin et al. 2000, Scheffer et al. 2001). Even in less abundant functional groups (e.g., nitrogen-fixers), the loss of critical traits that ensure ecosystem functioning and resilience may have important consequences (Grime 1998, Walker et al. 1999). The detection of such changes may be a hint of significant ecosystem transitions.

Species richness has traditionally been used to assess an ecosystem's response to environmental factors, and has been related to ecosystem multi-functionality (Maestre et al. 2012a). These relationships are largely modulated by other community attributes, such as species evenness and functional identity and divergence, which often respond more rapidly to environmental constraints than richness, and may have a strong impact on ecosystem processes (Chapin et al. 2000, Mouillot et al. 2011, Maestre et al. 2012b). Functional diversity, defined as the value, range, and relative abundance of the functional traits of biological communities in a given ecosystem, was shown to be a better and more universal predictor of ecosystem vulnerability than species diversity, which does not reflect the uneven role played by species in the maintenance of ecosystem processes (Tilman et al. 1997, Díaz et al. 2007). Functional diversity is usually assessed by the use of several metrics (e.g., community-weighted mean and functional richness, evenness, and divergence). Recent investigations have demonstrated the better predictive ability of indexes that consider species abundances rather than richness alone (Schleuter et al. 2010, Mouillot et al. 2011). However, there is no consensus on the best field method for functional diversity assessment. Biomass (Prieur-Richard et al. 2002), frequency (De Bello et al. 2005), and most commonly cover (Frenette-Dussault et al. 2012, Lavorel et al. 2008) are ordinarily used in the estimation of functional diversity. Nonetheless, cover estimates obtained by different methods may vary considerably (Abrahamson et al. 2011) and may therefore affect estimates of functional diversity.

The desirable characteristics of a cover sampling method to monitor functional diversity are primarily efficiency, precision, and reproducibility. Efficiency expresses the amount of information collected in relation to the resources devoted to achieve that

information. Precision, i.e., the bias between two measurements of the same object by the same observer, is essential to detect changes in the dominance of traits and to ensure measurement repeatability. The reproducibility of a method to be used by different observers largely depends on its objectivity, which is assumed to be higher for methods less vulnerable to the observer bias. The methods most commonly used for sampling plant cover are the area-based, modified-Whittaker's method (MW), and the point-intercept method (PT), based on transects (Goodall 1953, Stohlgren et al. 1995, Elzinga et al. 2001). The PT method was originally proposed for grasslands (Goodall 1953), and is based on the interception of species at predefined points along a transect. It is thus less biased than area-based methods, which rely on the visual assessment of plant cover (Elzinga et al. 2001). The line-intercept method is mainly used in patchy shrublands (De las Heras et al. 2011), but it is not suitable for species (e.g., grasses, some forbs, shrubs) with narrow or lacy canopies, whose extension is hard to delineate when plant density is high (Elzinga et al. 2001). Dengler's plot (DE - Dengler 2009) is an additional area-based MW-derived method recently proposed, but without the MW shortcomings of non-uniform plot sizes or shapes, nestedness, and spatial arrangement of smaller subplots. Although considerable attention has been devoted to comparisons of plant-sampling approaches over the last 20 years (Floyd & Anderson 1987), few studies have assessed the performance of different cover-sampling methodologies with respect to functional diversity (Abrahamson et al. 2011). For instance, the use of different methods may lead to a different number of species included in the 80% "dominance" threshold - thus affecting the "amount of trait diversity" analyzed - as proposed by Garnier et al. (2004) and Pakeman & Quested (2007) based on the mass-ratio hypothesis (Grime 1998).

With the aim of determining the best cover-sampling method for non-destructive fine-scale monitoring of functional diversity in semi-arid areas, we compared the abovementioned PT, MW, and DE methods in a vegetation survey carried out in Mediterranean Holm oak (*Quercus ilex* L.) woodland. Specifically, wet and dry locations were selected along a regional precipitation gradient to determine potential differences in methods' performance in relation to water scarcity. In the study area water deficit, often combined with low soil productivity, is the major limiting factor for plant establishment, thus lowering vegetation density and/or diversity. The following questions were addressed: (i) Are there differences in the efficiency of these methods in quantifying the relative abundances of species and traits? (ii) Does the efficiency of these methods vary depending on the amount of precipitation and, consequently, with changing vegetation density and/or diversity? (iii) Do the differences lead to different estimates of functional diversity metrics?

The above methods were compared in terms of time-efficiency and precision of cover estimates for individual species, and main genera and families as well. Also, we assessed the effect of their use on several functional structure and diversity metrics, namely, "community-weighted mean" and functional richness, evenness, and divergence (Garnier et al. 2004, Villéger et al. 2008, Laliberte & Legendre 2010). We hypothesized the PT method (less biased and more reproducible) would provide more precise cover estimates than the other abovementioned methods, allowing a better quantification of functional diversity over time by different observers and across different environmental conditions.

2.3 Material and Methods

Study sites

The study was performed in a Mediterranean Holm oak (*Quercus ilex* L.) woodland (montado) in southwestern Portugal. This semi-natural savanna-like ecosystem has been shaped by human use since long time. Dominant soils in the study area are poor and shallow lithosols, grazing is moderate to low, and agricultural activity has ceased in recent years. The climate ranges from dry sub-humid to semi-arid, with large inter-annual variation. The plant community consists of scattered Holm oak trees and an herbaceous understory dominated by annual grasses and forbs, with shrubland patches dominated by *Cistus ladanifer* L. Two sampling sites were selected approximately 100 km apart at the extremes of a regional precipitation gradient (Table 2.1). To ensure within-plot homogeneity, sampling was carried out in highly homogeneous grassland areas with no drainage lines or flooding surfaces, and included herbaceous species and sub-shrubs (chamaephytes).

Table 2.1. Main characteristics of the sites sampled in this study. Sources: Atlas Digital do Ambiente (2011), Nicolau (2002).

	Dry	Wet
Locality	Almodôvar, Beja	Montemor-o-Novo, Évora
Geographical coordinates	37.603066° -8.011351°	38.495000° -8.216198°
Annual average precipitation 1961–1990 (mm)	592	748
Precipitation coefficient of variation (%)	63	59
Annual average temperature 1931–1960 (ºC)	17.5	16.0
Altitude (m)	253	187

Sampling methods

In the spring 2011, an area of approximately 1000 m^2 was randomly selected at each sampling location (Table 2.1). The sampling designs depicted in Fig. 2.1 was superimposed as much as possible over the selected area. Data on the herbaceous community and bare soil cover were collected by two experienced botanists working together, and the survey time recorded, using the following methods (Table 2.2):

1. the modified-Whittaker (MW) method (Stohlgren et al. 1995), with consistent rectangular proportions and independent and non-overlapping subplots nested within the largest plot.

2. The Dengler's (DE) method (Dengler 2009), based on the MW method but with fully nested, square sampling units (each plot nested within the parent larger plot) and replicates of equally-distributed smaller subplots.

3. The point-intercept (PT) method (Elzinga et al. 2001), using six 20-m linear transects systematically located (41 points each, spaced every 50 cm). At each point, a rod of 5mm in diameter was stuck in the ground with a 90° angle. All plant species, naked soil, lichens, litter, etc., touching the rod were recorded, though only plant data were considered in the subsequent analysis. The same species was recorded only once at each point. Species and group cover were calculated as the proportion of points intercepted per transect.

Table 2.2. Description of the sampling design adopted for each of the three survey methods analyzed.

		Modified-Whittaker (MW)	Dengler(DE)	Point-intercept (PT)
Description		20 × 50 m plot with one 100-m^2, two 10-m^2, and ten 1-m^2 subplots	31,6 × 31,6 m plot with three 100 m^2, three 10 m^2, and six 1 m^2 subplots	six 20 m transects with 41 points each spaced every 0.5 m
Measurement method		Species presence in a 1000-m^2 plot; cover estimates in ten 1-m^2 subplots	Species presence in a 1000-m^2 plot; cover estimates in six 1-m^2 subplots	Cover and species presence measured by point interceptions
Area/ length	Cover	10 × 1 m^2	6 × 1 m^2	6 × 41 = 246 points
	Presence	1000 m^2	1000 m^2	6 × 41 = 246 points

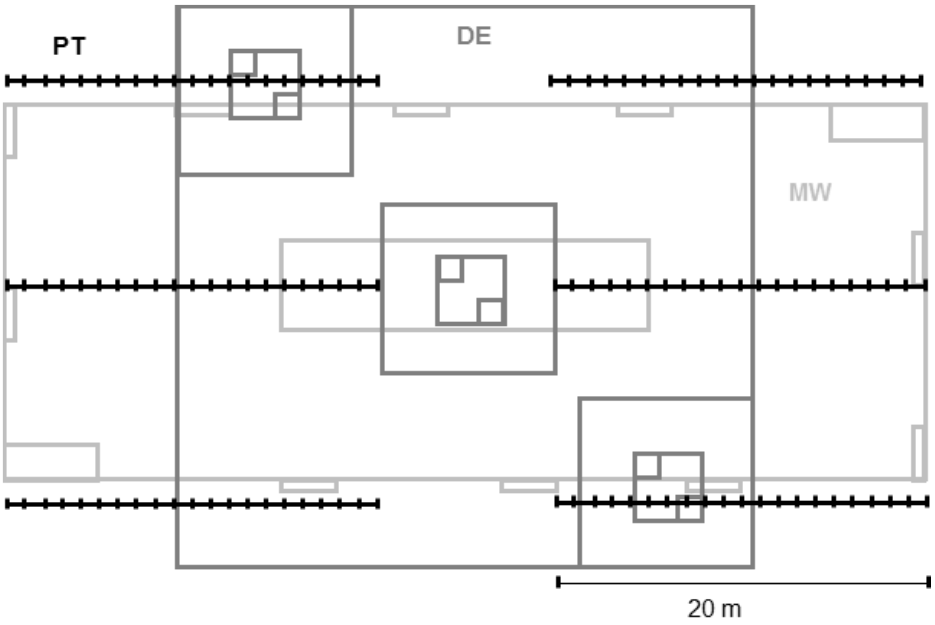

Figure 2.1. Sampling design and field overlaying schemes. (MW): modified Whittaker's plots (light gray); (DE): Dengler's plot (dark gray); (PT): point-intercept method (black).

Data analysis

Overall richness, number of botanical families, and Shannon's diversity index (Kent & Coker 1992) were calculated for each method at each location (mean of 1-m^2 subplots or transects). Pielou's evenness index was also determined (Pielou 1975).

Precision of cover estimates

The mean and precision of the cover estimates obtained with the three methods at the two locations were compared (data from 1-m^2 subplots or transects, $N \geqslant 6$) for species cumulatively attaining a relative cover $\geqslant$ 80%, as well as for genera cumulatively attaining a relative cover $\geqslant$ 50% and for the main botanical families, namely, Asteraceae, Fabaceae, and Poaceae (cumulatively attaining a relative cover $\geqslant$ 90%).

Functional traits

To compare functional structure and diversity estimates among methods, we selected eight traits reflecting the strategies used by species to cope with the main environmental constraints, i.e., water and soil-nutrient limitations. Binary, semi-quantitative,

and quantitative traits were considered, including growth-form, N-fixing ability, dispersal mode, life-cycle, flowering onset and duration, vegetative height at maturity, and specific leaf area (leaf area/dry weight - Table 2.3). These traits are related to stress and disturbance avoidance/tolerance, resource acquisition and retention, and reproductive and dispersal abilities (Cornelissen et al. 2003). Traits were assigned to each species (Table S2.1 in Appendix 2.1) based on either direct observations or literature reports (Franco 1971, Castro 2008, Porto et al. 2011, Bernard-Verdier et al. 2012). Growth form (graminoid or other) and dispersal mode (anemochorous or other) were coded as binary traits to reflect the most relevant characteristics in the grassy plant community (Table 2.3).

Table 2.3. Description of the functional traits considered in the study. For species trait assignments, see Table S2.1 (Appendix 2.1). Source: (1) direct observation/measurement; (2) Franco (1971); (3) Porto et al. (2011); (3) Castro (2008); (4) Bernard-Verdier et al. (2012).

Type	Functional trait	Categories/Units	Function	Source
Binary	Growth form	Graminoid	Disturbance avoidance and tolerance, decomposition rate	1,2
		No		
	N-fixing ability	Yes	Resource acquisition, nutrient cycling	1
		No		
	Dispersal	Anemochory	Dispersal distance	1,3
		Other		
Semi-quantitative	Life cycle	Annual Biennial Perennial	Stress and disturbance avoidance and tolerance	1,2,3
	Onset flowering	Initial month	Reproductive strategy, stress avoidance	2
	Flowering duration	Number of months		2
Quantitative	Height	cm	Light capture, competitive vigor, dispersal distance	1
	Specific leaf area	mm^2/mg	Photosynthetic rate, growth rate, leaf life span	3,4

Functional diversity metrics

To describe functional diversity, we used the indexes proposed by Villéger et al. (2008), who considered species within a community distributed in a multidimensional functional space. This approach has the advantage of taking into account species abundance and considering simultaneously several traits. Using these indexes, functional richness, functional evenness, and functional divergence were computed for the combined

eight traits listed in Table 2.3. A detailed description of the computational method is reported by Villéger et al. (2008). Functional richness is the amount or range of functional multidimensional space occupied by a community and is calculated based on the convex-hull volume method. Functional evenness reflects the regularity of the distribution of abundance in a trait space. A higher functional evenness is expected to correspond to a fuller occupation of a niche space by coexisting species (Mason et al. 2005). Functional divergence quantifies the functional dissimilarity of trait values within a community. For instance, divergence is high when the functional trait values of the most abundant species are far outside the center of the functional trait range (Villéger et al. 2008). High functional divergence can be used as an indicator of a high degree of niche differentiation and low competition for resources (Mason et al. 2005). Additionally, the functional dispersion (Laliberté & Legendre 2010), which is closely related to Rao's quadratic entropy, was calculated for all the traits combined. Functional dispersion is defined as the weighted-mean distance in multidimensional trait space of individual species from the weighted centroid of all species, using as weight the species' relative abundance. We also calculated the functional dispersion and functional evenness for each trait individually, as well as the "community-weighted mean" (CWM), proposed by Garnier et al. (2004), for the three methods at each location. CWM is defined as the average trait value in a community weighted by the relative abundances of the species carrying each value, and reflects the dominant traits in a community. All calculations were carried out using the "dbFD" function implemented in the FD package (Laliberté & Shipley 2011) and running under the R© environment (R Core Team 2013).

Statistical analysis

Separate tests were performed for each location, since site comparison was out of the scopes of this analysis (see above). Departure from normal distribution of data was tested by Kolmogorov-Smirnov tests. Kruskall-Wallis nonparametric tests were applied to test for differences among methods in individual species cover, cover of the main genera and families, and functional structure and diversity estimates. Multiple comparisons were carried out by pairwise Wilcoxon rank sum tests using Bonferroni's adjustments at $\alpha=0.05$. To remove variation due to mean effect size (Lewontin 1966), Levene's test for homogeneity of variances was applied on log-transformed species' cover values to detect significant differences in the precision of estimates. To compare the precision of cover estimates

among methods, the coefficient of variation (CV: ratio of standard deviation to mean) was analyzed with likelihood ratio tests (Verrill & Johnson 2007), thereby removing variation due to differences between mean cover values. Student's t-test was used to compare species diversity indexes. Nonparametric and Levene's tests were conducted using R© version 3.0.2 (R Core Team 2013).

2.4 Results

The average time needed for two people to complete the vegetation survey at dry and wet locations was 125 and 170 min, respectively, ranging from 93 to 207 min, with no considerable differences among the MW, DE, and PT methods (data not shown). The overall richness recorded with the MW method was higher than that determined using the DE method within 1000-m^2 plots. By contrast, in 1-m^2 plots or 20-m transects similar cumulative richness were obtained by the MW and PT methods, whereas fewer species were detected using the DE method (Table 2.4). At the wet location, the PT method revealed a higher diversity index and a higher evenness than either MW or DE methods (Table 2.4).

Table 2.4. Overall cumulative richness, number of botanical families, Shannon's diversity index (mean; N⩾6), and evenness, recorded at dry and wet locations, for each sampling method. (MW): modified-Whittaker's method; (DE): Dengler's method; (PT): point-intercept method. Values with different letters are significantly different across columns after Bonferroni's test adjusted at P<0.017 (N⩾6).

	Dry			Wet		
Methods	MW	DE	PT	MW	DE	PT
Within a 1000-m^2 plot						
Richness	40	34	–	79	63	–
Within 1-m^2 plots or transects						
Cumulative richness	26	17	26	48	36	45
Cumulative number of families	5	3	6	11	12	13
Richness	10 ± 2^{a}	11 ± 3^{ab}	16 ± 4^{b}	15 ± 3^{a}	14 ± 3^{a}	21 ± 2^{b}
Diversity	1.4±0.2	1.4±0.2	1.7±0.5	1.8 ± 0.4^{a}	2.1 ± 0.2^{a}	2.6 ± 0.1^{b}
Evenness	0.6±0.1	0.6±0.0	0.6±0.2	0.7 ± 0.1^{a}	0.8 ± 0.0^{b}	0.9 ± 0.0^{c}

At the species level, whenever differences were noted, the PT method generally yielded absolute cover estimates that were significantly higher than those obtained with the other two methods at both locations (Table 2.5). The PT method also revealed a lower CV both for more and for less abundant species at the wet location (e.g., *Vulpia myurus,*

Chamaemelum mixtum, Cerastium glomeratum) and for less abundant species at the dry location (*Lolium rigidum*). The number of species necessary to attain a relative cover of 80% (relative to the sum of all species cover) differed among the three methods, with consistently higher values using the PT method. At the dry location, the 80% threshold was reached with 8, 7 and 3 species using the PT, MW and DE methods, respectively. At the wet location, these differences were even higher: 17, 10, and 7 species for the PT, DE, and MW methods, respectively (Table 2.5).

Table 2.5. Absolute mean cover (%) and coefficient of variation (CV) recorded for the most common species (attaining ⩾80% relative cover) by each method (MW: modified-Whittaker; DE: Dengler; PT: point-intercept) at dry and wet locations. Values with different letters are significantly different across columns after Bonferroni's test adjusted P<0.017 (N ⩾ 6). Lowercase letters refer to cover comparison and uppercase letters to CV comparison. Poaceae species: *Agrostis pourretii, Chaetopogon fasciculatus, Gaudinia fragilis, Holcus annuus, Vulpia myuros, Lolium rigidum, Bromus lanceolatus*; Asteraceae species: *Chamaemelum mixtum, Carlina racemosa, Crepis vesicaria, Tolpis barbata, Leontodon taraxacoides*; Fabaceae species: *Ornithopus compressus, Trifolium campestre, Trifolium cernuum, Trifolium glomeratum, Trifolium striatum*; Caryophyllaceae species: *Cerastium glomeratum*. Species cumulatively attaining ⩾80% of relative cover with each method are highlighted in bold.

	Dry						Wet					
Methods	MW		DE		PT		MW		DE		PT	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Poaceae												
Apour	**67.0**	0.2	**80.0**	0.2	**76.4**	0.1	**45.0**	0.6	**16.7**	0.8	**30.9**	0.6
Cfasc	**7.5**	3.4	1.6	0.9	**8.9**	1.5	0.1	5.8			**4.5**	2.2
Gfrag	**6.8**^{a}	0.6	5.7^{a}	0.4	**32.5**^{b}	0.4	**5.0**	1.2^{AB}	**8.8**	1.2^{A}	**18.3**	0.3^{B}
Hannu	**14.8**	1.0	8.9	0.2	**24.4**	0.5	**3.7**	4.4^{A}	1.7	0.0^{B}	**2.0**	0.9^{AB}
Vmyur	**9.0**^{a}	1.3	**28.3**^{ab}	0.5	**46.3**^{b}	0.2	2.4	1.5^{A}	**6.8**	2.0^{AB}	**10.6**	1.0^{B}
Lrigi	0.2	1.7^{A}	0.1	0.0^{B}	2.0	0.9^{B}	1.6	2.3	1.3	1.3	**18.3**	1.0
Blanc	0.2	1.3	0.1	0.0	2.8	1.4	0.4	1.1	1.2	3.3	**0.4**	2.4
Asteraceae												
Cmixt	0.2	0.9	0.8	0.8	**3.7**	0.9	**13.7**	1.4^{AB}	**12.0**	1.5^{A}	**8.5**	0.5^{B}
Crace	**17.6**	0.7	**15.8**	0.5	**26.8**	0.2						
Cvesi	**4.9**^{a}	3.2	1.2^{ab}	1.3	**8.9**^{b}	0.5	**5.2**^{a}	0.7	**11.8**^{ab}	0.7	**22.8**^{b}	0.5
Tbarb			0.2	0.0	0.4	2.4	**11.0**	0.8	**18.8**	0.4	**17.9**	0.6
Ltara					0.4	2.4	0.2^{a}	6.4^{A}	**1.9**^{ab}	0.9^{AB}	**2.8**^{b}	0.4^{B}
Fabaceae												
Ocomp	0.9	4.8	0.8	2.5	3.7	0.9	1.9	0.8	**4.5**	0.8	**5.3**	1.0
Tcamp	1.7	2.4	0.6	0.7	2.4	1.5	1.1	2.1	1.2	1.8	**1.2**	1.7
Tcern							2.1	5.3	0.1		**6.5**	1.5
Tglom							**5.9**	1.6	**7.0**	1.1	**5.3**	0.8
Tstri							2.7	1.2	**2.8**	0.8	**4.9**	1.2
Caryophyl.												
Cglom							0.4	5.4^{A}	0.2	1.2^{A}	**2.0**	0.9^{B}

Whenever there were differences in the cover of the main genera and botanical families, significantly higher estimates and lower CVs were obtained with the PT method than with the other methods, particularly at the dry location (Table 2.6). Cover estimates for Poaceae species were highest using the PT method, at both locations. At the dry location, the PT method yielded cover values for Asteraceae family species and for the genus *Vulpia* higher than those obtained with the DE and MW methods, respectively, while at the wet location estimates for Fabaceae species with PT were higher than with MW (Tab. 6). The precision of the cover estimates was significantly higher for estimates obtained with PT (lower CV) than with MW for the genera *Agrostis* and *Vulpia* at the dry location, and for Asteraceae species at both locations (Table 2.6).

Table 2.6. Absolute mean cover (%) and coefficient of variation (CV) recorded for the main genera (attaining >50% relative cover) and families (attaining >90% relative cover) for each method (MW: modified-Whittaker; DE: Dengler; PT: point-intercept) at dry and wet locations. Values with different letters are significantly different across columns after Bonferroni's test adjusted at P<0.017 (N⩾6). Lowercase letters refer to cover comparison and uppercase letters to CV comparison.

Sampling methods	Dry						Wet					
	MW		DE		PT		MW		DE		PT	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Main genus												
Agrostis	67.0	0.4^{A}	80.0	0.2^{B}	76.4	0.1^{B}	45.0	0.7	16.7	1.6	30.9	0.6
Chamaem.	0.3	1.0	0.8	1.0	3.7	0.9	13.7	1.4	12.0	1.5	8.5	0.5
Tolpis	0.0		0.2	2.5	0.4	2.5	11.0	0.9	18.8	0.4	17.9	0.6
Trifolium	1.8	1.8	0.6	0.8	2.8	1.4	15.0	0.7	12.9	0.9	25.2	0.6
Vulpia	9.0^{a}	1.2^{A}	28.3^{ab}	0.5^{AB}	46.3^{b}	0.2^{B}	2.4	1.4	6.8	2.0	10.6	1.0
Main families												
Asteraceae	39.3^{ab}	0.6^{A}	19.4^{a}	0.3^{AB}	53.7^{b}	0.2^{B}	30.7	0.6^{A}	44.6	0.4^{AB}	54.9	0.3^{B}
Fabaceae	3.2	1.1	1.9	1.0	8.1	0.8	17.1^{a}	0.6	18.7^{ab}	0.7	35.0^{b}	0.4
Poaceae	107.3^{a}	0.2	125.7^{ab}	0.2	207.7^{b}	0.1	59.0^{ab}	0.4	37.2^{a}	0.4	90.7^{b}	0.3

Of the functional metrics performed for the combined eight traits, only functional evenness differed significantly among the methods used, with higher estimates for the PT method (Fig. 2.2).

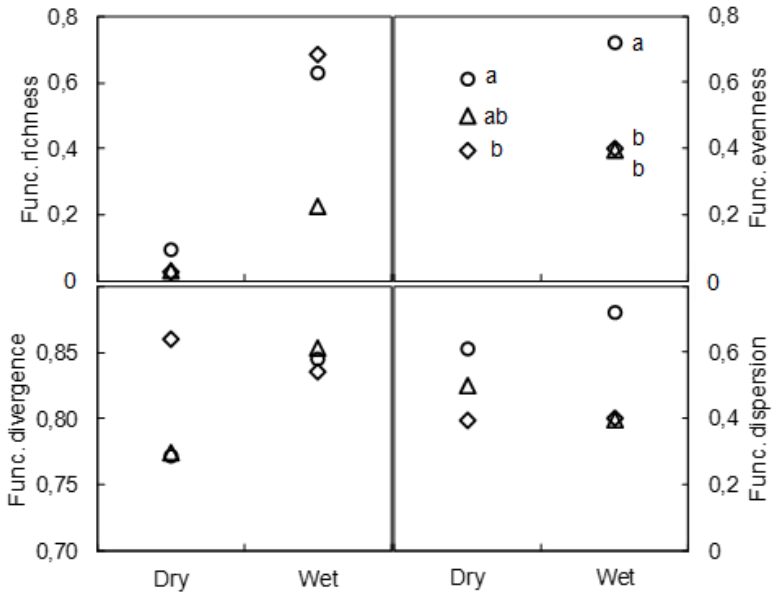

Figure 2.2. Overall functional diversity indices (8 traits) at dry and wet locations. Functional richness, functional evenness, functional divergence (Villéger et al. 2008), and functional dispersion (Laliberté & Legendre 2010). Methods: modified-Whittaker (MW, diamonds); Dengler (DE, triangles); point-intercept (PT, circles). Different letters indicate significant differences between methods after Bonferroni's test adjusted $P<0.017$ ($N \geqslant 6$).

Functional metrics computed individually for the four binary, semi-quantitative, and quantitative traits are shown in Fig. 2.3. The results for the other four traits are provided in Fig. S2.1 (Appendix 2.1). The DE method resulted in lower CWM values for N-fixing ability at both locations and in higher estimates of functional dispersion than those provided by either PT or MW at the dry location (Fig. 2.3). The CWM of flowering onset was lower using PT than DE at both locations, whereas functional evenness estimates with DE were higher than those obtained with either other methods at the wet location. Height functional evenness was higher with PT than with DE at the wet location, whereas the functional dispersion of specific leaf area differed with each of the three methods at both dry (DE>PT) and wet (DE>MW) locations (Fig. 2.3).

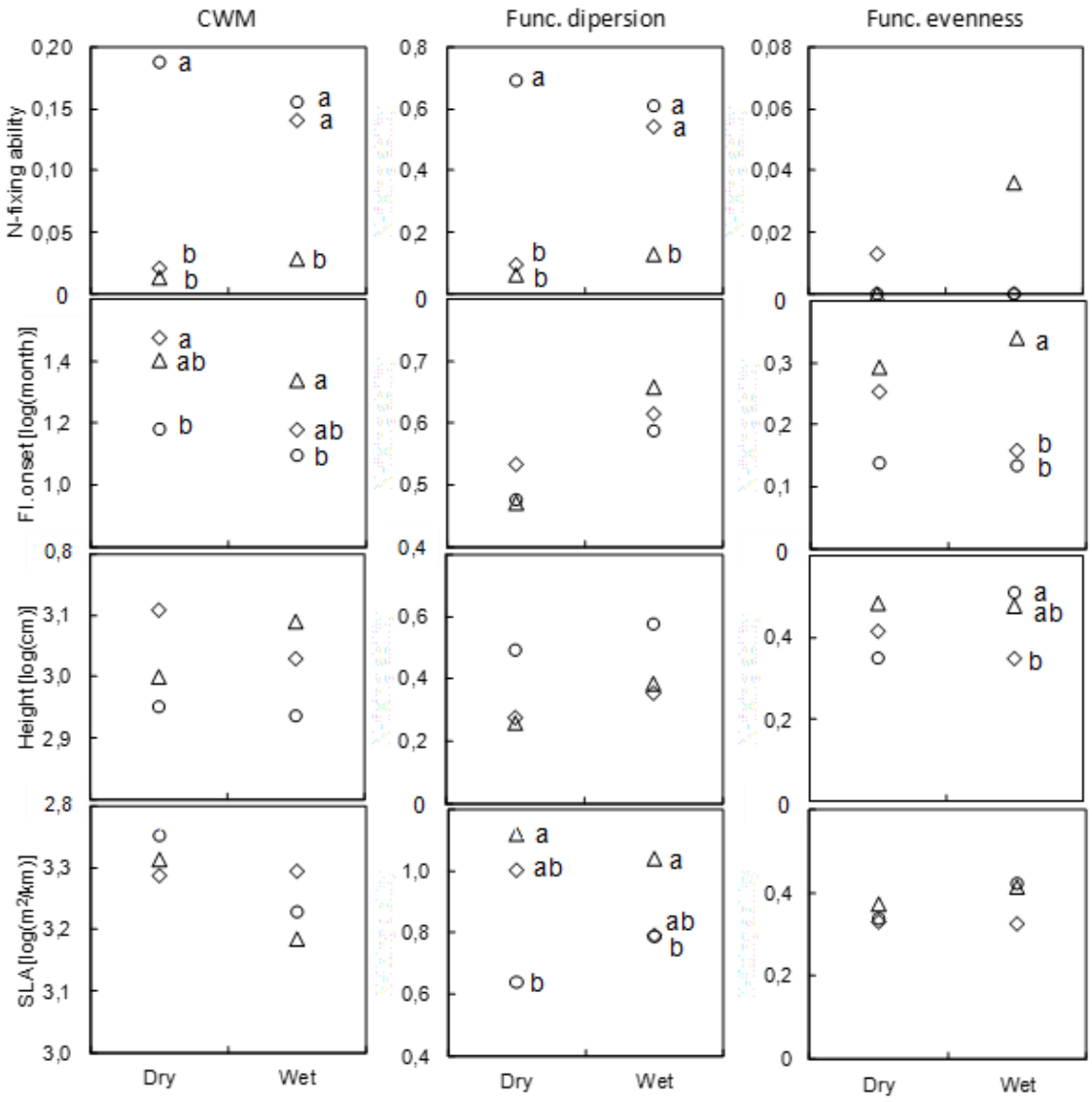

Figure 2.3. Community weighted mean (CWM), functional evenness, and functional dispersion in dry and wet locations. Traits: N-fixing ability (binary), onset of flowering (semi-quantitative), and height and specific leaf area (SLA - quantitative). Methods: modified- Whittaker (MW, diamonds); Dengler (DE, triangles); point-intercept (PT, circles). Different letters indicate significant differences between methods after Bonferroni's test adjusted at $P<0.017$ ($N \geqslant 6$).

2.5 Discussion

In this study, three methods commonly used in the field to quantify species/traits showed remarkable differences of efficiency, leading to divergent estimates of the "community-weighted" functional diversity metrics, regardless of the trait values themselves. This "field sampling-method effect" on functional diversity estimates has relevance in plant functional ecology, especially when the aim of the study implies a fine-

scale survey. Indeed, a number of studies relied on plant abundance measured in the field to weight trait importance in the computation of functional metrics, while collecting at least some of the species trait values from bibliographic sources or databases (Fischer et al. 2013, Gerhold et al. 2013). Our results showed that the field method chosen in the survey affects community weighted means, functional evenness, and functional divergence. All such parameters are required to achieve a reliable assessment of the functional structure and diversity of plant communities.

Although no differences among the three methods were found in the time needed for the survey, the observed differences in their efficiency in quantifying the species/traits abundance point out important advantages of the PT method in non-destructive fine-scale monitoring of plant functional diversity in semi-arid areas. Firstly, this method allowed the detection of as many species as the MW method and of a higher number than the DE method. It also provided a higher precision in cover estimates and, because of higher community evenness, more species/traits could be analyzed within the advocated 80% “dominance” threshold (species representing 80% of the relative cover of the community). Therefore, the PT method offers a cost-effective way to reduce “sampling error”, by reducing the subjectivity in species cover estimates and improving functional diversity estimates. Secondly, this method is less vulnerable to the operator’s bias and thus likely more reproducible when used by different operators (Elzinga et al. 2001). It is worth to stress that we did not aim at testing the effect of different observers, e.g., through ring tests to assess the “observer error” (Giordani et al. 2009). Instead, we assumed a priori that the reproducibility of the PT method is higher (lower observer-bias) when used by experienced botanists familiar with the local flora.

The PT method has proven to be more efficient across contrasting environments (different precipitation regimes) and showed consistency even across different plant density or diversity. These features are critical for a precise and reproducible assessment of changes in functional diversity of plant community in response to environmental changes. In this study, the PT method outperformed other methods commonly used, for fine-scale monitoring of plant functional diversity in the understory of Holm-oak woodlands. Similarly, we hypothesized its superior performances also in vegetation surveys of semi-arid areas characterized by low tree density and/or dominated by grasslands and/or shrubland patches. However, further analyses are needed before extending our results to other ecosystems like boreal or temperate forests.

Species quantification and richness

With the PT and MW methods, a higher number of species was quantified than with the DE method, i.e., PT and MW performed better in species/traits quantification. This is a critical step in functional diversity assessments. Even though our study was not aimed at testing methods to assess species richness *per se*, we found that using the two area-based methods considered (MW and DE) a higher overall number of species was detected, as a consequence of the inclusion of an extra 1000-m^2 plot for the detection of "new" species presence. However, if species richness is an index of interest in the survey, the PT method could be easily complemented with a search for new species in a predefined surrounding area with little extra time.

Precision of cover estimates

The PT method adopted in this study provided plant cover estimates more precise than those obtained with the other methods tested. At the dry location, major differences were found for main genera and families. Cover estimates by the PT method were shown to be particularly sensitive to plant architecture and leaf morphology of understory plants (Abrahamson et al. 2011). However, such method performed similarly or even better than others in monitoring groups of species with distinct morphology (e.g., Asteraceae), ensuring at the same time similar or higher precision in the assessment of cover by species group as well. At the wet location, precision differences concerned mainly individual species' estimates, either dominant or not, with a consistent advantage of the PT method in the case of varying plant density or diversity. In accordance with our results, other authors using the PT method in herbaceous communities, have reported a precision similar or higher than that obtained by cover visual estimates (Vittoz & Guisan 2007). Precision in plant abundance quantification is an essential prerequisite of a reproducible method suitable to fine-scale monitoring of functional diversity of herbaceous communities, where changes in species abundance may be hard to detect (e.g., dense/rich communities).

Evenness and quantification of less abundant species

In the wet location analyzed, community evenness estimates obtained with the PT method were higher than those resulted from the application of the other methods, with small differences in relative cover from more to less abundant species. Likely, such differences were underlying the divergence of functional evenness estimates among the three methods considered. Community evenness plays an important role in ecosystem

processes and multifunctionality (Hillebrand et al. 2008, Maestre et al. 2012a). Functional evenness reflects the distribution of traits in a community and thus the degree of niche space occupation by coexisting species (Mason et al. 2005). It often responds more rapidly to environmental changes than species richness and may have a rapid and strong impact on ecosystem functions (Chapin et al. 2000, Mouillot et al. 2011). Area-based methods implying visual cover estimates (MW and DE) tend to overestimate the dominant and more conspicuous species and underestimate those less abundant (Vittoz & Guisan 2007), with accordingly lower evenness. This was the case at the wet location, where seven species attained 80% relative cover according to the MW method, while seventeen species were needed to achieve the same threshold with the PT method.

It is important to take into account the role of less common species in functional diversity assessments. A threshold of 80% (in terms of species number or their cover) has been advocated as representative in the description of functional composition (Garnier et al. 2004, Pakeman & Quested 2007). Such threshold is based on the assumption that dominant species are functionally more important because of their higher abundance, following the mass-ratio hypothesis (Grime 1998). Thus, since the adoption of the PT method included more species (and more functional traits) within the above threshold, it follows that PT performed better for purposes of assessing functional diversity. Moreover, the role of less common species in drought-prone ecosystems may be critical, both over the short- and the long-term. In drylands, inter-annual climatic fluctuations are high, forcing species to cope with extreme values of the environmental factors. Consequently, they often exhibit a dynamic turnover involving shifts in the abundance of response groups when a rainy year is followed by a severely dry one, especially in communities dominated by annual species (Aronson & Shmida 1992, Adler & Levine 2007, Elmendorf & Harrison 2009). Therefore, in contrast to more mesic areas, less abundant species in drylands are likely to play a major role in the ecosystem resilience, as a consequence of their capability of exploiting outstanding environmental conditions, as proposed by the complementarity hypothesis (Grime 1998, Walker et al. 1999, Loreau 2000). This argument supports the relative importance of less abundant species and thus their inclusion in functional diversity assessments. In a study on grasslands by McIntyre & Lavorel (2001), the range of different traits exhibited by forbs and smaller grasses contributed to a varying environmental response, in contrast to local dominant grasses. In our study, this seemed to be the case of N-fixing species. Despite the fact that their relative cover did not exceed 3.0 % and 17.6 % at the dry and wet locations, respectively, they represented a highly relevant functional feature of drylands, usually characterized by soil N shortage (Sprent & Gehlot 2010).

Furthermore, drylands are highly susceptible to land degradation and desertification (Reynolds et al. 2007). These processes most likely depend on a critical threshold beyond which drastic alterations occur, preceded by more subtle functional changes in communities (Reynolds et al. 2007, Scheffer et al. 2001). It is therefore important to monitor such changes, as they represent early-warning indicators and allow a timely adoption of counteracting prevention activities.

Cover estimates

Absolute cover estimates were generally higher with the PT method than with either other methods, as previously reported in the literature (Abrahamson et al. 2011). This effect has been attributed to the error due to the diameter of the rod used in sampling, which should be as thin as possible (Elzinga et al. 2001). However, this is not a problem when the aim is to monitor changes through time and it should not affect the computation of functional metrics using the relative cover of species/traits.

2.6 Conclusions

In this study, we demonstrated that the PT, MW, and DE methods differed in terms of efficiency in the assessment of species/traits relative abundances, thus affecting estimates of functional diversity. The PT method had important advantages over the others with respect to fine-scale monitoring of plant functional diversity in the mainly grassy understory of Holm oak woodlands, and likely in semi-arid areas in general as well. The adoption of the PT method allowed the detection of as many species as the MW method and of more species than the DE method, with a higher precision of cover estimates both for groups of species and at the single-species level. Moreover, due to higher community evenness, it allowed the analysis of a greater number of species/traits within the advocated 80% “dominance” threshold. Precision of the estimates is a prerequisite of functional diversity surveys, in that they must include not only dominant species and traits, because of their larger contribution to ecosystem functionality, but also less common ones, given their decisive role in the resilience and function of semi-arid ecosystems, thereby integrating the mass ratio and complementarity hypotheses (Loreau 2000). The advantages of the PT method were proven across contrasting environmental conditions. In addition, it should be less biased than those based on visual estimation and thus more reproducible when used by different operators.

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Supporting Information

Table S2.1. List of species recorded and trait category/value assigned (excluding specimens not identified to species level). Nomenclature follows Flora Iberica (Castroviejo et al. 1986–1999). Traits: Gram – graminoid growth form; N – N fixing ability; Lcyc – life cycle; Hg - height (cm); Bflow – onset of flowering (month); Dflow – duration of flowering (number of months); Anem – Anemochorous dispersal mode; SLA – specific leaf area (mm^2/mg).

Species	Gram	N	Lcyc	Hg	Bflow	Dflow	Anem	SLA
Daucus carota	no	no	peren	11.9	5	2	no	17.7
Andryala intergrifolia	no	no	peren	26.3	3	10	no	NA
Carlina corimbosa	no	no	peren	21.4	6	4	yes	15.3
Carlina racemosa	no	no	biennial	16.4	8	3	yes	14.5
Chamaemelum fuscatum	no	no	annual	7.2	10	8	yes	NA
Chamaemelum mixtum	no	no	annual	28.2	2	10	yes	20.8
Crepis capillaris	no	no	biennial	19.3	3	4	yes	28.1
Crepis vesicaria	no	no	biennial	33.6	2	5	yes	18.8
Hypochaeris glabra	no	no	annual	12.7	2	4	yes	34.1
Leontodon taraxacoides	no	no	annual	17.1	3	4	yes	18.0
Pulicaria odora	no	no	peren	36.5	5	7	yes	NA
Pulicaria paludosa	no	no	biennial	36.5	5	7	yes	NA
Tolpis barbata	no	no	annual	20.5	4	5	no	35.3

Species	Gram	N	Lcyc	Hg	Bflow	Dflow	Anem	SLA
Echium plantagineum	no	no	biennial	33.8	2	5	no	32.9
Campanula lusitanica	no	no	annual	15.7	4	4	no	NA
Cerastium glomeratum	no	no	annual	5.2	1	6	no	21.8
Illecebrum verticillatum	no	no	annual	2.9	2	5	no	57.9
Polycarpon tetraphyllum	no	no	annual	4.8	4	4	no	NA
Silene gallica	no	no	annual	15.7	2	9	yes	18.8
Spergula arvensis	no	no	annual	7.2	2	4	no	15.0
Tuberaria guttata	no	no	annual	16.2	2	6	no	21.6
Capsella bursa-pastoris	no	no	annual	40.0	12	6	yes	NA
Sisymbrium officinale	no	no	annual	24.1	4	4	no	26.7
Lotus corniculatus	no	yes	peren	8.7	3	5	no	21.8
Lotus hispidus	no	yes	annual	7.5	NA	NA	no	NA
Lotus parviflorus	no	yes	annual	8.7	4	3	no	NA
Medicago polymorpha	no	yes	annual	11.1	3	5	no	20.1
Ornithopus compressus	no	yes	annual	19.8	2	5	no	33.1
Trifolium angustifolium	no	yes	annual	16.9	3	6	no	19.6
Trifolium bocconei	no	yes	annual	6.7	5	2	no	23.3
Trifolium campestre	no	yes	annual	8.6	3	7	no	41.6
Trifolium cernuum	no	yes	annual	4.5	6	1	no	NA
Trifolium cherleri	no	yes	annual	4.7	3	5	no	24.4
Trifolium dubium	no	yes	annual	NA	4	3	no	26.6
Trifolium glomeratum	no	yes	annual	9.6	3	4	no	26.7
Trifolium repens	no	yes	peren	12.5	3	10	no	28.2
Trifolium striatum	no	yes	annual	17.2	4	3	no	20.9
Trifolium subterraneum	no	yes	annual	9.4	2	5	no	25.0
Vicia benghalensis	no	yes	annual	16.4	4	3	no	22.9
Exaculum pusillum	no	no	annual	6.5	7	2	no	NA
Erodium botrys	no	no	annual	5.4	2	4	no	NA
Geranium rotundifolium	no	no	annual	4.2	3	4	no	23.9
Isoetes sp.	no	no	peren	NA	2	4	no	NA
Juncus buffonius	yes	no	annual	3.0	3	7	yes	17.9
Mentha pulegium	no	no	peren	45.0	6	5	no	NA
Urginea maritima	no	no	peren	30.0	9	2	no	NA
Lythrum junceum	no	no	peren	NA	4	3	no	NA

Species	Gram	N	Lcyc	Hg	Bflow	Dflow	Anem	SLA
Plantago coronopus	no	no	biennial	13.1	2	5	yes	19.6
Plantago lagopus	no	no	peren	16.9	3	3	yes	21.3
Plantago lanceolata	no	no	peren	22.4	4	3	yes	18.9
Agrostis pourretii	yes	no	annual	21.9	4	4	yes	36.7
Avena barbata	yes	no	annual	61.1	2	5	yes	25.4
Brachypodium distachyon	yes	no	annual	18.1	3	4	yes	32.8
Briza maxima	yes	no	annual	24.9	3	5	yes	35.8
Briza minor	yes	no	annual	6.0	3	5	yes	NA
Bromus hordeaceus	yes	no	annual	17.8	3	4	yes	28.2
Bromus lanceolatus	yes	no	peren	21.0	4	3	yes	26.8
Bromus rubens	yes	no	annual	9.7	4	2	yes	NA
Chaetopogon fasciculatus	yes	no	annual	19.2	4	3	yes	NA
Cynosurus echinatus	yes	no	annual	NA	4	4	yes	NA
Gastridium ventricosum	yes	no	annual	14.2	4	5	yes	NA
Gaudinia fragilis	yes	no	annual	27.8	3	4	yes	20.2
Holcus annuus	yes	no	annual	25.9	6	2	yes	NA
Lolium rigidum	yes	no	annual	28.3	3	6	yes	25.3
Molineriella laevis	yes	no	annual	10.2	3	3	yes	56.0
Phalaris coerulescens	yes	no	peren	30.0	4	4	yes	NA
Taeniatherum caput-medusae	yes	no	annual	33.8	4	2	yes	20.0
Triticum aestivum	yes	no	annual	NA	NA	NA	yes	22.0
Vulpia myuros	yes	no	annual	14.1	3	4	yes	18.1
Rumex acetosella	no	no	peren	22.7	3	6	yes	14.1
Rumex bucephalophorus	no	no	annual	11.5	2	5	yes	NA
Anagallis arvensis	no	no	annual	12.1	3	4	no	29.2
Galium aparine	no	no	annual	NA	3	5	no	35.7
Galium parisiense	no	no	annual	9.5	4	4	no	24.5
Linaria spartea	no	no	annual	50.0	3	4	no	NA

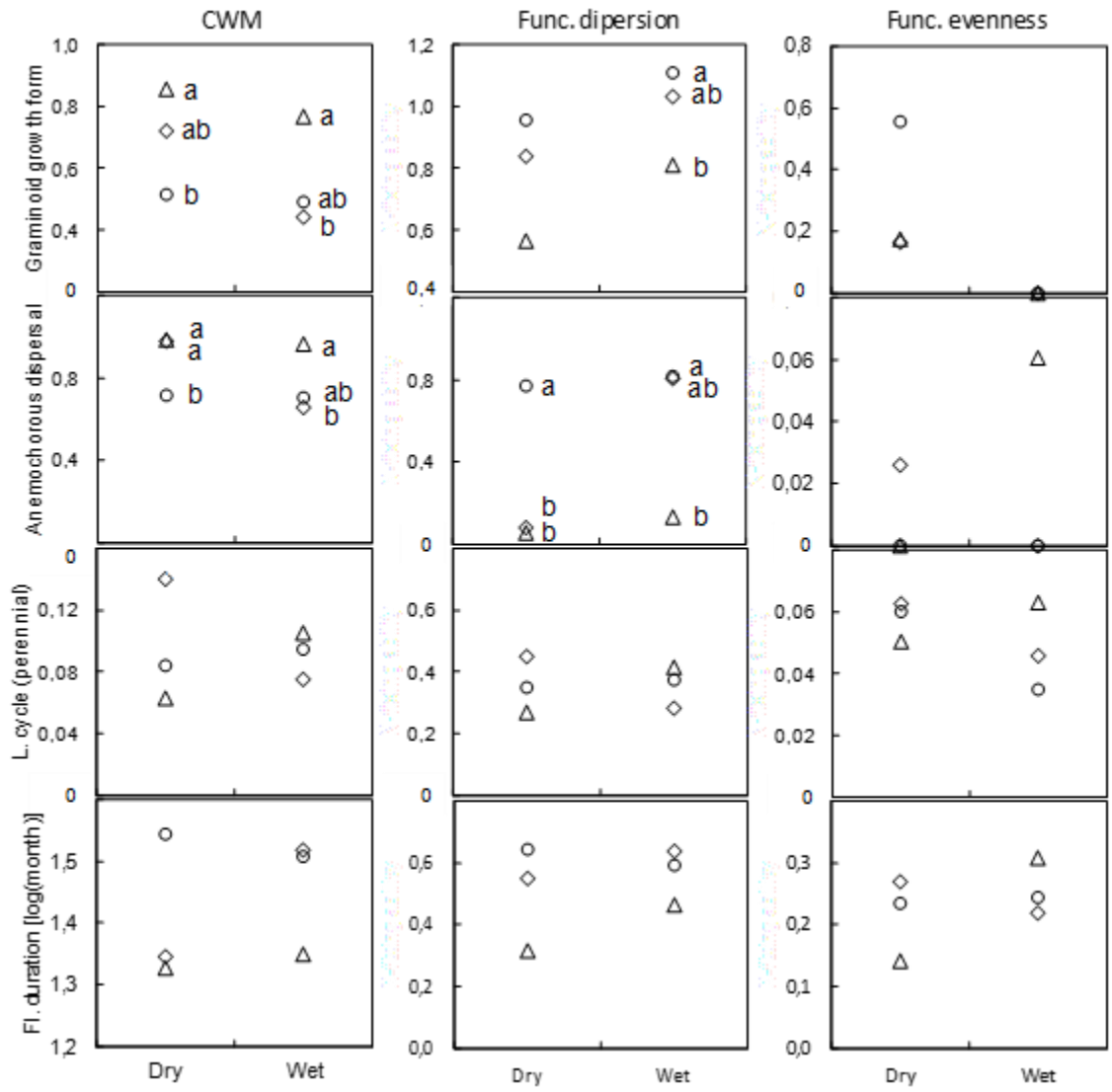

Figure S2.1. Community-weighted mean (CWM) (Garnier et al. 2004), functional evenness (Villéger et al. 2008), and functional dispersion (Laliberté & Legendre 2010) for graminoid growth form and anemochorous dispersal mode (binary traits), life cycle, and flowering duration (semi-quantitative traits), in dry and wet locations. Methods: modified-Whittaker (diamonds); Dengler (triangles); point-intercept (circles). Different letters indicate significant differences between methods for a Bonferroni's adjusted $P<0.017$ ($N\geq6$).

Chapter 3

Which plant traits respond to aridity? A critical step to assess functional diversity in Mediterranean drylands

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3 Which plant traits respond to aridity? A critical step to assess functional diversity in Mediterranean drylands

3.1 Abstract

Aridity acts as a strong environmental filter to plants, limiting major ecosystem processes. Climate change models predict an overall increase of aridity in drylands. This could lead to changes in plant communities, particularly in the dominance and range of plant functional traits, which largely determine ecosystem functioning. However, to study how changes in aridity may affect plant functional metrics, a critical decision needs to be taken: the choice of the functional traits to be studied. Previous studies related plant functional traits and aridity, however mostly focusing on a single facet of functional diversity and primarily on perennial species. Hence, the response of plant traits to aridity quantifying different functional metrics at the whole-community level (considering also annual species) is not well established in drylands. Here, we use a high-resolution aridity gradient along a Mediterranean dryland ecosystem of Holm-oak woodlands to identify plant functional traits responding to aridity at the community-level (comprising annual and perennial species). We studied how the community-weighted-mean and functional dispersion of 13 traits related to plant establishment, growth, reproduction, dispersal and persistence changed with aridity. Nine plant functional traits varied with aridity. Aridity acted as an environmental filter on community-weighted-means, increasing the dominance of annual species, particularly rosettes, and plants with lower maximum height, shorter flowering duration, and increased anemochorous dispersal. Higher aridity was associated to an overall decrease in functional dispersion, particularly for life cycle, specific leaf area, onset of flowering, dispersal strategy and seed persistence traits, probably due to a lower niche differentiation under more arid conditions. The changes in community-weighted-means and in functional dispersion due to aridity are likely to negatively affect major ecosystem functions such as biomass production and nutrient cycling. Our results fill an important knowledge gap by quantifying how the functional structure and dispersion of 13 plant traits change with aridity at the whole-community level, providing an important basis for the selection of key functional traits to be used in trait-based studies in drylands.

Keywords: annual plants; climatic gradient; community-weighted-mean; functional dispersion; grassland; Holm-oak woodlands

3.2 Introduction

The study of functional traits of organisms in addition to a solely taxonomic approach to diversity is becoming more and more common in ecological studies (Díaz et al., 2016; Kunstler et al., 2016). This is because functional traits may be linked to ecosystem functions, thus providing a more mechanistic understanding of species response to environmental factors, and can also help predict the effect of species on ecosystem processes (e.g., primary productivity, nutrient cycling) (Cadotte et al., 2011; Lavorel and Garnier, 2002; Mason and de Bello, 2013; Tobner et al., 2014). The functional structure and diversity of a community are most commonly defined as the mean value and range of species functional traits in that community (Díaz et al. 2007a; Lavorel et al. 2008).

Whatever the objective of the use of functional diversity may be, there is a critical decision to be taken: the choice of the functional traits to be studied at the community-level. Traits should be selected according to their responsiveness to a certain factor or to their effect on ecosystem processes. Measuring species traits is often laborious and time consuming, hence many trait-based studies addressed at the community-level rely on trait data retrieved from scientific literature or trait databases (Díaz et al., 2016; Kattge et al., 2011; Kunstler et al., 2016). Many functional traits may not vary independently but rather co-vary, reflecting patterns of resource allocation or trade-offs among plant strategies, and different combinations of traits may be adopted by species under different environments to maximize their performance (Maire et al., 2013; Volis and Bohrer, 2013; Costa-Saura et al., 2016; Díaz et al., 2016). However, to assess which functional traits are affected by a certain driver and check if and how they co-vary, it is indispensable to begin with an individual evaluation of each trait.

The functional traits of a biological community are most commonly described by two complementary community-level metrics (Díaz et al. 2007a; Lavorel et al. 2008). One is functional structure or the so-called community-weighted-mean (CWM), which reflects the dominant traits in a community (Garnier et al., 2007). It is based on the mass ratio hypothesis, according to which dominant species exert a key effect on ecosystems (Grime, 1998). CWM enables to quantify community shifts in mean trait values due to environmental selection for certain functional traits, associated to the replacement of dominant or subdominant species with particular traits by other(s) with different traits. The other component is trait range or functional dispersion (FDis), which reflects the degree of functional dissimilarity among the community, and can be expressed through various metrics (Laliberté and Legendre, 2010; Mason et al., 2005; Villeger et al., 2008).

Functional dispersion may be used to quantify the decrease or increase in trait dissimilarity along ecological gradients compared to a random expectation (i.e. trait convergence or divergence, respectively). Communities characterized by a high functional dispersion lead to a more complete utilization of resources, and thus to increased ecosystem functions, such as biomass accumulation or decomposition, according to the niche complementary hypothesis (Tilman et al., 1997; Tobner et al., 2014). Both community-level functional metrics (CWM and FDis) were reported to respond to major environmental filters such as climate, disturbance regime or biotic interactions, and to affect major ecosystem processes like primary productivity or decomposition rates (Díaz et al., 2007a; De Bello et al., 2010; Mouillot et al., 2011; Mouillot et al., 2013; Valencia et al., 2015).

Aridity acts as a strong environmental filter on plant communities (e.g. determining species presence/absence) and limits major ecosystem processes such as primary productivity and nutrient cycling (Delgado-Baquerizo et al., 2013). Climate change predictions point to an overall increase of aridity in drylands worldwide (Dai, 2013; IPCC, 2007). This could lead to changes in vegetation structure and composition and alter the dominant plant traits (CWM) and the FDis of dryland communities. Increasing aridity may change the dominance of distinct life-forms in the community (Noy-Meir, 1973), and favor particular growth-forms (e.g. grasses, species with a prostrate habit) (Fay et al., 2002) that may confer higher ability to withstand dry periods. Also, aridity may select for smaller species (Gross et al., 2013), as a strategy to reduce the risk of cavitation under increased water stress (Enquist, 2002). Aridity may select for an earlier flowering onset and shorter flowering duration (Hänel and Tielbörger, 2015; Kigel et al., 2011), as a way to reduce the risk of reproductive failure as the dry season progresses. Aridity may also affect dispersal strategies and promote higher seed mass and seed longevity (Baker, 1972; Arroyo et al., 2006; Metz et al., 2010; Arellano and Peco, 2012), thus increasing the chances of survival and persistence under dry environments (Volis and Bohrer, 2013; Gremer and Venable, 2014). Increasing aridity may favor species with a particular type of root system, more able to maximize water and nutrients acquisition during short peaks of resource availability (Schenk and Jackson, 2002) or, on the contrary, promote the coexistence of a wide variety of root systems, capable of a more complete resource utilization. Finally, for perennial species, aridity may favor stress-tolerant strategies e.g. evergreen leaves with low specific leaf area (SLA) (Ackerly et al., 2002; Costa-Saura et al., 2016). However, in the most arid conditions these species can be replaced by short-lived stress-avoidant species with semi-deciduous leaves and high SLA (Ackerly et al., 2002; Gross et al., 2013). In addition

to possible changes in trait dominance, aridity may also modify community FDis of particular traits, by selecting a wider or narrower variety of coexisting functional strategies or trait values. A previous work found a higher than expected functional dispersion of leaf traits within dryland communities, attributing it to the high specialization of the flora to drought adaptation (Freschet et al. 2011). Contrastingly, other work found decreased functional dispersion of particular traits in response to aridity (e.g. plant height), suggesting that different traits may respond differently to aridity (Gross et al., 2013). Changes in community FDis may have important consequences for ecosystem functioning and resilience. This is because a high FDis is expected to lead to improved ecosystem functioning (Díaz et al., 2007a; Mouillot et al., 2011; Tobner et al., 2014). In addition, high FDis has been hypothesized to increase the resilience of dryland ecosystems to aridity (Volaire et al., 2014), because it would enhance the chances that some species could survive under more arid conditions, thus maintaining ecosystem functioning (Díaz et al., 2007a).

Previous studies have tried to examine the relationship between plant functional traits and aridity. Although all of them contributed to partially clarify this subject, there is no clear picture about which are the main plant traits responding to aridity, particularly at the whole-community level. The main limitations of previous studies are: (i) addressing simultaneously other environmental factors interacting with aridity (Adler et al., 2004); (ii) studying only one component of functional traits, e.g. the CWM (Barboni et al., 2004); (iii) using low-resolution gradients e.g. no more than five sites under different aridity levels (De Bello et al., 2005; Frenette-Dussault et al., 2012; Lavorel et al., 2011); (iv) studying aridity impacts on particular (few) species (Hänel and Tielbörger, 2015) or only on perennial species (Gross et al., 2013; Valencia et al., 2015; Costa-Saura et al., 2016), not considering the whole plant community. The latter reason is a major shortcoming, considering that annual species constitute an important part of the diversity of dryland plant communities (Aronson et al., 1993; Noy-Meir, 1973). In addition, annuals play a critical role in ecosystem functioning, e.g. in energy flow and nutrient cycling (Baldocchi et al., 2004; Gilliam, 2007; Ramos et al., 2015). They have a faster species turnover than perennials, and thus are expected to respond more readily to environmental changes. To our knowledge, for many plant traits it remains unclear whether their community-weighted-mean and functional dispersion respond simultaneously to aridity (i.e., if both change), and, if so, if their response is similar (e.g. increasing or decreasing). Clarification could be improved if works contemplated a higher number of plant traits addressed at the whole-community level along aridity gradients with higher resolution.

In this paper, we propose to identify plant functional traits responding to aridity in a Mediterranean dryland ecosystem. To do so, we evaluated simultaneously functional structure and functional dispersion at the community-level (comprising annual and perennial species), along a spatial gradient of aridity with high-resolution. We studied 13 traits related to species establishment, growth, reproduction, dispersal and persistence. We expect aridity to act as an environmental filter of species traits from an available regional pool, affecting (i) the functional structure of the community (i.e. the mean value of traits); (ii) traits' functional range, by selecting for a narrower variety of plant strategies highly adapted to aridity and to climatic variability found in drylands (i.e. increasing the similarity of the trait values of co-existing species). We tested the following hypotheses: (i) CWM and FDis of each particular trait will show different trends along the aridity gradient; (ii) single-trait and multi-trait FDis will decrease with increasing aridity, due to environmental filtering.

3.3 Material and methods

Study sites and the aridity gradient

This study was carried out in southwestern Iberian Peninsula, Europe. Field data were collected in 54 Mediterranean dryland sites dominated by savanna-like Holm-oak woodlands (*Quercus ilex* L. subsp. *rotundifolia*) along a regional aridity gradient, comprising semi-arid and dry sub-humid climates (Fig. 3.1). We used the aridity index (AI) of the United Nations (Middleton and Thomas, 1992) representing the ratio of mean annual precipitation to annual potential evapotranspiration to describe the aridity gradient. AI data for the period 1950–2000 were retrieved from a global aridity database (http://www.cgiar-csi.org/data/global-aridity-and-pet-database) (Trabucco and Zomer, 2009), and ranged from 0.42 to 0.56 along the sampling sites. Mean annual precipitation (50 yrs. average) along the study area varied from 520 mm to 634 mm, with a considerable inter annual variation (the annual precipitation at each site can vary by more than 100 mm between years). Sampling took place in a relatively dry year (370±61 mm) when compared to the 50-year mean annual precipitation of the sampling sites altogether (561±27 mm).

The selection of the sampling sites was based on circular plots (250 m radius) surveyed for the National Forest Inventory 2005/06 (AFN, 2010). From the ca. 336000 plots occupied by Holm-oak woodlands, a pre-selection of sites was made to avoid

confounding effects of factors other than aridity and ensure homogenization as much as possible. We selected sites with an altitude between 150 and 300 m a.s.l., soil dominantly acidic (pH<6.5) dominated by sedimentary and metamorphic lithology, and with no fire records (6242 plots) (AFN, 2010; Atlas Digital do Ambiente, 2011). Afterwards, a random selection of sites stratified by the aridity index was made, to ensure an even representation of different aridity levels. We ended up with 54 sampling sites, distributed along two main groups in space (North and South), although not very far apart (Fig. 3.1). We tested the importance of this 'position variable' as an additional predictor and, because it was not relevant for the functional response of the plant community to aridity (i.e. not significant in the models), we discarded it from further analyses. In addition to the previously described characteristics, the sampling sites had moderate to low grazing intensity and no agricultural activities over the last five years. To check the latter conditions, we inspected evidence of grazing in vegetation, the amount of ungulate pellets, and absence of recent soil tillage in the field. We sampled the understory of Holm-oak woodlands with a sparse tree cover (mean < 40 trees per ha) (Amaral et al., 1997), consisting of variable proportions of semi-natural grasslands intermingled with shrubland patches (shrub cover ranging from 0% to 87%, average 16%).

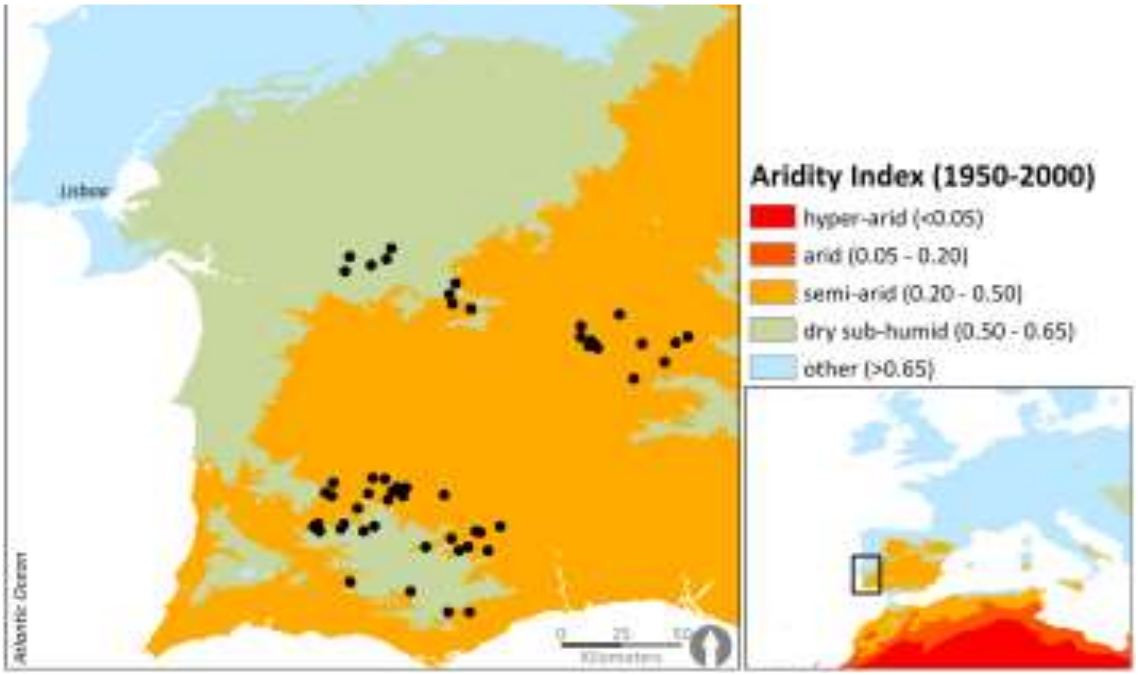

Figure 3.1. Map with the location of the study sites along the aridity gradient evaluated.

Vegetation sampling

Plant sampling was conducted at the peak of standing biomass (April-June 2012) using the point–intercept method, avoiding drainage lines and flooding surfaces. At each site, species cover was estimated as the proportion of points intercepted along six 20 m transects systematically arranged over an area of ca. 1000 m2 and 10 m apart from each other, with points spaced every 50 cm (246 points per site) (for further detail on the sampling scheme see Nunes et al., 2014). At each point, a rod of 5 mm in diameter was stuck to the ground with a 90° angle. All plant species touching the rod were recorded and the same species was recorded only once at each point. Species cover was calculated as the proportion of points intercepted along the six transects.

Trait selection and functional metrics calculation

We found 236 species along the entire gradient and each sampling site had a mean of 35±12 species. Because it was not possible to obtain trait data for all the species found due to time and resource limitations, we relied on the most abundant species, which are expected to have most of the influence on ecosystem functioning, following the mass ratio hypothesis (Grime, 1998). We used the dominant species attaining at least 80% of the relative cover, because it is considered an adequate proportion to characterize functionally a plant community (Pakeman and Quested, 2007). We used species attaining this threshold in decreasing order of relative cover within single sites (95 species overall, and 30±9 per site) to compute functional diversity metrics. We selected 13 plant traits including continuous, ordinal, categorical and binary traits, reflecting plant strategies related to establishment, persistence, regeneration, and dispersal (Table 3.1). Trait information was obtained either through direct measurements in the field following standard protocols (Pérez-Harguindeguy et al., 2013), or derived from various bibliographic sources (see Appendix A in Supporting Information), or both (Table 3.1). A single trait value per species was used to compute functional diversity metrics, because we were focused on the turnover between sites and not on intraspecific trait variability.

To inspect the functional structure of the plant community, the community-weighted-mean (CWM) was calculated for each trait. It corresponds to the average trait value in a community weighted by the relative abundance of the species carrying each value (Garnier et al., 2007). While for continuous traits CWM values represent the mean value of that trait in the community, for categorical and binary traits CWM values correspond to the proportion of each category (or functional group) in the community, i.e.

their relative abundance. To examine trait range we used functional dispersion (FDis). It measures the degree of functional dissimilarity within the plant community and is closely related to Rao's quadratic entropy (Laliberté and Legendre, 2010). It is calculated as the weighted mean distance, in multidimensional trait space, of individual species from the weighted centroid of all species, where weights correspond to species relative abundances (Laliberté and Legendre, 2010). The minimum value FDis can take is 0 (e.g. communities composed of only one species) and it has no upper limit. FDis has several advantages over other indices that measure functional dissimilarity: it takes into account species relative abundances, it is unaffected by species richness, can handle any number and type of traits and is not strongly influenced by outliers (Laliberté and Legendre, 2010). We calculated FDis for each trait and also for groups of traits (multi-trait FDis). Multi-trait FDis was computed for (i) all 13 traits; (ii) for the five traits more correlated with aridity; (iii) for the leaf-height-seed (LHS) traits involved in the 'plant strategy scheme' proposed by Westoby (1998). We calculated multi-trait FDis attributing the same weight to all traits (unweighted) and also attributing different weights to traits, giving a lower weight to traits with a high correlation between them. Continuous traits were log transformed prior to analysis. The Gower distance was used in calculations because it can handle continuous, ordinal, and categorical variables (a kind of trait standardization), as well as missing values. All calculations were done with the dbFD function of the FD package (Laliberté et al., 2014) in R (R core Team, 2015).

Data Analysis

For all the analysis performed, aridity represents 1 - Aridity Index, so that higher aridity values correspond to drier conditions, to facilitate results interpretation. We tested for the significance of correlations between single-trait (CWM and FDis) and multi-trait (FDis) functional metrics and aridity (1 - Aridity Index). We used Spearman correlations to account for possible nonlinear relationships. Overall, we tested for the significance of correlations for 27 CWM variables, 13 single-trait FDis variables, and 6 multi-trait FDis variables (correlations were considered significant for $P < 0.05$). In addition, we also tested for the significance of Pearson correlations among the single-trait functional metrics (CWM and FDis) calculated for each trait (Tables S3.2 and S3.3). All the analysis were performed under R statistical environment (R coreTeam, 2015).

3.4 Results

Mean functional structure of the plant community

In general, the plant community was dominated by therophytes (76%) and by graminoid plants (50%) (Table 3.1). The mean shrub cover was 16% but varied greatly among sites (SD=25%) and was mostly composed of species with semi-deciduous leaves (92%). Average maximum plant height was 24 cm and mean specific leaf area (SLA) was 20.9 mm2/mg. Flowering started on average in March (CWM=3) and lasted 4 months, despite some variation among sites. Most species were dispersed by wind (74%) or by gravity (23%), whereas only a minority of the plant species exhibited zoochorous dispersal (3%). Mean seed mass was 0.38 mg and most species exhibit short seed persistence between 1 and 5 years (69%) (Table 3.1). On average, only 5% of the community had the ability to fix N and average maximum root depth was 0.40 m (Table 3.1).

Plant traits and aridity

Nine of the CWM and five of the FDis community values were significantly related to aridity (Table 3.1); four traits showed no significant association with aridity, namely leaf type of woody species, seed mass, N-fixing ability and root depth.

Regarding the functional structure of the plant community (CWM), more arid conditions were negatively associated with perennial life cycle ($p<0.001$) and this was also reflected, in a negative though less strong correlation with phanerophytes ($p<0.05$) and with shrub growth-form ($p<0.05$) (Table 3.1). We found significantly lower plant height under drier conditions ($p<0.01$). Additionally, the plant community at drier sites showed shorter flowering duration ($p<0.05$) and less species with barochorous dispersal ($p<0.001$), while anemochorous dispersal exhibited the opposite trend ($p<0.01$) (Table 3.1). A positive correlation with aridity was observed for short-lived therophytes ($p<0.01$), as well as for rosette growth-form ($p<0.05$) (Table 3.1).

Functional dispersion (FDis) was lower in drier sites for plant life cycle ($p<0.01$), SLA ($p<0.01$), onset of flowering ($p<0.05$), dispersal strategy ($p<0.01$) and seed persistence ($p<0.05$) traits, evidenced through a negative correlation with aridity (Table 3.1). Multi-trait FDis computed jointly for these five traits, and also for the 13 traits all together was negatively correlated with aridity ($p<0.05$ and $p<0.01$, respectively), both with the traits unweighted and weighed (Table 3.2, Table S3.4).

Table 3.1. CWM and functional dispersion (FDis) values computed for each trait (mean of all sites ± standard deviation). Spearman correlations (ρ) between these metrics and aridity (1- Aridity index) (n=54 sites). See Table S3.1 for more information on functional traits. The mean values of continuous and semi-quantitative traits are in the original scales. Leaf type trait was characterized only for woody species, which were present only in 38 sampling sites. P-value: *<0.05; **<0.01; ***<0.001.

			Functional structure (CWM)		Functional dispersion (FDis)	
Trait	Type	Categories*/units*	Mean ± SD	Spearman ρ	Mean ± SD	Spearman ρ
Vegetative						
Life cycle	Ordinal		0.19 ± 0.25	**-0.43*** **	0.48 ± 0.44	**-0.44****
Life-form	Categorical	Therophyte	0.76 ± 0.24	**0.36****	0.21 ± 0.06	-0.14
		Hemicryptophyte	0.05 ± 0.07	-0.21		
		Geophyte	0.00 ± 0.02	0.07		
		Chamaephyte	0.01 ± 0.05	-0.25		
		Phanerophyte	0.15 ± 0.23	**-0.31***		
Growth-form	Categorical	Bulb	0.00 ± 0.02	0.07	0.28 ± 0.05	-0.18
		Erect	0.12 ± 0.09	-0.21		
		Graminoid	0.50 ± 0.16	0.24		
		Prostrate	0.04 ± 0.04	0.10		
		Rosette	0.17 ± 0.12	**0.31***		
		Shrub	0.16 ± 0.25	**-0.34***		
Max. height	Continuous	*cm*	24.10 ± 1.52	**-0.41****	0.50 ± 0.18	-0.10
Leaf						
SLA	Continuous	*mm^2/mg*	20.90 ± 1.44	0.20	0.70 ± 0.30	**-0.39****
Leaf type	Categorical	Evergreen	0.00 ± 0.03	-0.27	0.05 ± 0.11	-0.11
		Semi-deciduous	0.92 ± 0.20	-0.12		
		Green-stemmed	0.07 ± 0.20	0.13		
Reproductive						
Onset flower.	Semi-quant.	*initial month*	3.04 ± 1.11	-0.21	0.50 ± 0.23	**-0.27***
Duration flower.	Semi-quant.	*nr. of months*	4.17 ± 1.11	**-0.37****	0.52 ± 0.21	-0.20
Regenerative						
Dispersal strategy	Categorical	Anemochory	0.74 ± 0.22	**0.36****	0.21 ± 0.09	**-0.35****
		Barochory	0.23 ± 0.22	**-0.45*** **		
		Ectozoochory	0.02 ± 0.03	0.13		
		Endozoochory	0.01 ± 0.02	0.09		
Seed mass	Continuous	*mg*	0.38 ± 1.80	0.25	0.66 ± 0.15	-0.20
Seed persistence	Ordinal	Long	0.13 ± 0.18	-0.14	0.50 ± 0.24	**-0.32***
		Short	0.69 ± 0.18			
		Transient	0.15 ± 0.09			
Below-ground						
N-fixing ability	Binary		0.05 ± 0.05	-0.04	0.25 ± 0.23	-0.04
Max. root depth	Continuous	*m*	0.39 ± 1.42	-0.23	0.65 ± 0.24	-0.23

Table 3.2. Spearman correlations between aridity (1- Aridity index) and multi-trait functional dispersion (FDis) metrics computed for: (i) all 13 traits; (ii) for five traits significantly correlated with aridity (life cycle, SLA, dispersal strategy, onset of flowering, seed persistence); (iii) for LHS traits (SLA, máx. height and seed mass). Multi-trait metrics were computed for traits unweighted (all traits with weight equal to 1), and for traits with different weights (see Table S3.4). P-value: ´<0.1; *<0.05; **<0.01; ***<0.001. N=54 sites.

	Traits unweighted	Traits with different weights
All traits (13)	-0.42**	-0.45**
Traits correlated with aridity (5)	-0.37*	-0.37*
LHS traits (3)	-0.25´	-0.24´

3.5 Discussion

Our results showed that aridity greatly influenced the plant community by affecting most of the functional traits studied. We found changes in functional structure (CWM) and in trait dispersion (FDis) in the response of the plant community to aridity. These findings support the important role of both functional metrics in the response of communities to different environmental factors, as suggested by several authors (De Bello et al., 2010; Dias et al., 2013; Tobner et al., 2014). Aridity acted as a strong environmental filter causing shifts in mean trait values along the gradient. These shifts were associated with changes in species abundances and possibly also with the replacement of some species by others exhibiting different traits. In addition, we found an overall lower FDis in drier sites, indicating a higher functional similarity among co-existing species. This trend was particularly evident for five traits. Aridity seemed to affect species recruitment from the regional pool, probably due to a reduction in the number of available niches for plants under more arid conditions (Ricotta and Moretti, 2011). Overall, nine of the 13 traits studied responded to aridity either through changes in their mean values and/or range along the aridity gradient, which are likely to affect several ecosystem functions.

Aridity affected the functional structure

Aridity operated as an environmental filter selecting for traits related to key plant strategies to cope with severe and often unpredictable drought, typical of the intra and inter-annual climatic fluctuations occurring in drylands. The CWMs of all vegetative traits that reflect plant survival and growth adaptation strategies to deal with drought, were related to aridity. This was particularly evident for life cycle, life-form, and máx. height. Plant communities from drier sites had lower abundance of perennial species and a

correspondent increase in annual species (therophytes). This may imply lower soil protection, particularly in dry years when annuals may fail to germinate, leaving the soil more susceptible to surface runoff and erosion. Short-lived species have in general higher photosynthetic rates and higher leaf turnover than perennials (Garnier et al., 1997), and may therefore play an important role in annual biomass production (Ramos et al., 2015) and in nutrient cycling (Kazakou et al., 2006). The negative association found between plant height and aridity may be partially due to the increase in the proportion of annual plants at drier sites. Lower plant stature may be a strategy to reduce the risk of cavitation under increased water stress (Enquist, 2002). It may also result from a lower aboveground competition (for light) in drier sites when compared to more mesic ones (Petru et al., 2006), which is known to favor taller plants (Pérez-Harguindeguy et al., 2013). The increase in rosettes (plants with a basal rosette near the soil) with aridity, although showing a weak correlation, may be explained by some advantages of this life-form under drought. Prostrate leaves from rosettes seem to allow the plant to reduce water losses by benefiting from CO2 derived from soil for photosynthesis, and from an improved microclimate for growth (Cramer et al., 2007). In addition, it may also provide a higher resistance to physical damage from herbivory and trampling in drier sites (Díaz et al., 2007b).

While we found no association between the leaf type of woody species and aridity, a previous study performed in Mediterranean shrublands reported a dominance of species with deciduous leaves at the drier extreme of an aridity gradient comprising 12 sites (Gross et al., 2013). In our case, the shrub component of the plant community was in general dominated by semi-deciduous species, and this might have prevented the emergence of such trend. In previous studies with Mediterranean plant communities, SLA (i.e. the ratio of leaf area to dry mass) was shown to decrease with aridity, due to the dominance of drought-tolerant species (Ackerly et al., 2002; Costa-Saura et al., 2016). Yet, we found no consistent changes in community mean SLA values with aridity. The reason for such difference may be because previous studies were based exclusively on perennial species (Ackerly et al., 2002; Costa-Saura et al., 2016), while we took into account also the contribution of annuals to community SLA mean values.

Aridity led to a shorter duration of flowering. This agrees with a previous work reporting a reduction in the growing season length in more arid sites in comparison with wetter sites along a similar gradient of aridity (Ramos et al., 2015). On one hand, the concentration of reproduction events on shorter favorable periods may be an advantageous strategy to ensure the continuity of the species under unpredictable arid environments (Petru et al., 2006). On the other hand, it means less time for pollination

to occur, affecting biotic interactions with pollinators and interconnected ecosystem processes, hence likely reducing the delivery of this service (de Bello et al., 2010). The tendency for an earlier onset of flowering has been observed for some annual species grown under higher aridity levels, when compared to those from less arid sites (Hänel and Tielbörger, 2015; Kigel et al., 2011). This trend was regarded as an adaptation to arid environments by stress-avoidance, diminishing the risk of early death before seed production (Hänel and Tielbörger, 2015; Kigel et al., 2011). However, no consistent trend was found for flowering onset in geophytes and perennial grasses at the more arid sites (Kigel et al., 2011), suggesting that this strategy is not widespread in the plant community. This may explain why we found no relation between onset of flowering and aridity at the whole community level, i.e. considering both annual and perennial species.

Aridity also affected plant dispersal strategies, favoring anemochory mostly at the expense of barochory. By promoting dispersion at longer distances and independent from animals, this strategy increases the chances of encountering more favorable conditions in space during prolonged drought periods, as a way to adapt to unpredictable environments (Volis and Bohrer, 2013; Gremer and Venable, 2014).

The mean value of seed traits (seed mass and seed persistence) of the plant communities studied did not change consistently with aridity. These findings contrast with the classical ecological theory according to which species from climatically unpredictable and more arid ecosystems should exhibit a higher proportion of persistent seeds as a bet-hedging strategy, and produce larger seeds, than species from more predictable mesic ecosystems (Baker, 1972; Gremer and Venable, 2014). Larger seeds provide increased nutrition and are expected to develop into larger seedlings with increased chances of survival, particularly in arid environments (e.g. Moles and Westoby, 2006; Metz et al., 2010), although this may not always be the case (Arellano and Peco, 2012). While some works support these theoretical predictions, others found the opposite trend, i.e. decreasing seed mass and dormancy of annual plants with increasing aridity (Harel et al. 2011). It has been suggested that plant reproductive strategies under arid environments involve a trade-off between seed persistence, seed size and dispersal strategies (Venable & Brown 1988). Large seed size would help to withstand unfavorable conditions, while seed dormancy and dispersal at longer distances would help to escape them (in time or in space, respectively) (Venable & Brown 1988). This suggests that alternative strategies to cope with aridity may co-exist within a community, e.g. species with dispersal over longer distances and lower seed mass coexist with others with higher seed mass and dispersion at shorter distances. Moreover, multiple co-optimal combinations of these traits may be selected for a specific environment (Volis and

Bohrer, 2013). This would prevent the emergence of a clear trend in community-mean of seed traits in response to aridity, as happened in our study. It is important to stress that our sampling took place during a considerably dry year. Hence, we cannot exclude the possibility that the intense drought experienced may have prevented the germination of part of the seed bank, particularly of species with higher persistence, thus contributing to the lack of association found with aridity.

A previous work reported a decrease in absolute rooting depths with increasing aridity (Schenk and Jackson, 2002). The authors suggested that species with shallower root systems may take better advantage of small rainfall pulses, than deeper rooted species, particularly those lacking superficial roots (Schenk and Jackson, 2002; Schwinning and Sala, 2004). However, those results obtained at a larger spatial scale (comprising deserts, semi-deserts, scrublands, grasslands and shrub- and tree-savannas) may not be comparable to our regional aridity gradient, which does not include such contrasting situations. In our case, no association was found between root depth and aridity, suggesting that a variety of root systems co-exist in the plant community, enabling plants to obtain water at different soil depths, despite changes in aridity.

A recent work suggests that the maintenance of N-fixing ability in plants globally is not only related with direct ecological and/or climatic constraints (e.g. N-fixing plants should experience a competitive advantage over non-fixers in nitrogen-poor soils), but also with interactions between individual fixation strategies (facultative or obligate) and with the indirect influence of climate on the nitrogen cycle over evolutionary time (Sheffer et al., 2015). These findings may explain why we found no clear association between N-fixing ability and aridity.

Functional dispersion decreased with aridity

Functional dispersion decreased in response to aridity. This trend was particularly evident for five traits, and supported by multi-trait FDis metrics computed for different combinations of traits. At drier sites, more stressful conditions caused a selection of a narrower variety of plant strategies, probably due to a lower niche differentiation; as such, species were functionally more similar, sharing traits adapted to aridity.

Higher aridity led to a lower diversity of life cycle strategies within the plant community. Consequently, while in more mesic sites co-domination of species with different life cycles probably increased the functions of soil protection, biomass production, carbon sequestration and nutrient cycling, the plant community at drier sites may not be able to provide the same level of these functions. The same trend was

observed for SLA. Higher variability in specific leaf area in less arid sites indicates the co-existence of plants with contrasting leaf strategies commonly found in Mediterranean ecosystems i.e. stress-tolerant strategies and stress-avoidance strategies (Chaves et al., 2002; Werner et al., 1999). A higher diversity of leaf strategies entails a higher diversity of photosynthetic and growth rates, leaf longevity, litter decomposability, etc., among the plant community. Hence, a higher SLA diversity in wetter sites may have positive effects on ecosystem processes such as productivity and nutrient retention (Tilman et al., 1997), as opposed to drier sites where species tend to have more similar SLA values.

Higher aridity was associated with a lower FDis of plant dispersal strategies, and a similar trend was found for seed persistence, although with a weaker correlation. These traits reflect key mechanisms determining the capacity of species to disperse in space (dispersal strategy) and in time (seed bank persistence), and hence to persist in the community under arid and highly variable conditions in space and time (e.g. precipitation distribution). Many species can persist in the soil in the form of seed during long dry periods, sometimes for several years (Peco et al., 2003), waiting to germinate only when favorable climatic conditions return.

Climate largely affects plant phenological events (e.g. Peñuelas et al., 2004). In less arid sites, precipitation events and more favorable temperatures may last longer, thus allowing the coexistence of species with different flowering strategies. At drier sites, on the contrary, flowering onset should coincide with shorter favorable periods following rainfall events to succeed. This may explain why the plant communities in less arid sites showed a higher diversity in flowering onset, although this trait FDis and aridity were only weakly correlated.

A lower FDis, by narrowing the range of strategies adopted by plants, might reduce the resilience of dryland ecosystem to environmental change, as hypothesized by Volaire et al., (2014), at least for most traits. This is because it would reduce the chances that some species could survive under the new conditions, and thus maintain ecosystem functioning (Díaz et al., 2007a). A high FDis is expected to reflect a high complementary in resource use between species, therefore improving ecosystem functioning (Díaz et al., 2007a; Mouillot et al., 2011). As a consequence, a lower functional dispersion in drier sites most likely implies a reduction in ecosystem functions, as observed in other studies (Valencia et al., 2015) and also in species interactions related to the ‘missing’ plant trait values or categories (functional groups).

Our findings also show that CWM and FDis were affected by aridity, indicating that both metrics are important in the response of the plant community to aridity. CWM and FDis describe two complementary metrics: the mean and the dispersion of functional traits within a given community (Ricotta and Moretti, 2011). As a consequence, they are mathematically related, showing some interdependence. When CWM approaches the upper and lower limits of the trait range, FDis will necessarily decrease because only species with similar trait values (high or low, respectively) will be present, leading to a hump-shaped relationship between CWM and FDis (Ricotta and Moretti, 2011; Dias et al., 2013). Therefore, in many cases, CWM and FDis are significantly correlated, as happened with our data (Tables B2 and B3). Nevertheless, they convey different (complementary) information, except for binary traits, for which CWM and FDis have the same meaning. For some traits aridity affected only the CWM (life-form, growth-form, height, duration of flowering); for others it affected only FDis (SLA, onset of flowering, seed persistence); and for other traits it affected both CWM and FDis (life cycle, dispersal strategy). Our results emphasize their complementary and the importance of considering and integrating both to better understand the functional response of the plant community to aridity.

Finally, our aridity gradient, despite its high-resolution, lacked extreme levels of aridity. Our findings must therefore be evaluated in light of this limitation, e.g. when extrapolating our conclusions to plant communities subjected to higher aridity. It is also important to notice that we did not consider intra-specific variability, which might have a non-negligible role in the response of some traits to aridity (Siefert et al., 2015).

3.6 Conclusions

Despite previous studies dealing with functional traits and aridity, to our knowledge the response of plant traits to aridity at the whole-community level (including also annual species) quantifying different components of functional diversity remained understudied. Our work contributes to fill this knowledge gap, by quantifying changes in functional structure and in functional dispersion for 13 plant traits along a high-resolution aridity gradient. We were able to identify nine plant traits responding to aridity, providing an important basis for the selection of key traits in future trait-based studies in drylands.

Aridity affected the mean value (CWM) of six traits and the functional range (FDis) of five traits, thus highlighting the importance of both components as complementary functional metrics, and the need to consider both to fully understand the functional

response of the plant community to aridity. The observed changes in CWMs with aridity most likely affect ecosystem functioning (e.g. biomass production, nutrient cycling) and may also affect biotic interactions, e.g. with pollinators. Functional dispersion decreased with increasing aridity, leading to a higher functional similarity among species at drier sites. This is probably due to a lower degree of niche differentiation under more arid conditions, suggesting a lower complementarity in resource use between species and, consequently, a reduction in ecosystem functioning. In addition, it may reduce ecosystem resilience, as it reduces the chances that some species might survive and maintain ecosystem functioning, if environmental conditions change.

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Supporting Information

Appendix A. List of the bibliographic sources used for trait data.

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Appendix B.

Table S3.1. Description of the plant functional traits studied. The sources used to obtain trait information are indicated by superscript numbers: [1]directly observed or measured in the field following standard protocols (Pérez-Harguindeguy, et al. 2013); [2]derived from various bibliographic sources (see Appendix S1); [3]obtained both through direct measurement in the field and derived from bibliographic sources.

Type	Trait	Type	Categories/units	Function
Vegetative	Life cycle[3]	ordinal	Annual (0), biennial (0.5) or perennial (1)	Soil protection, biomass production, nutrient cycling, resistance to disturbance
	Life-form[3]	categorical	Therophyte (annual and facultative biennial), hemicryptophyte, geophyte, chamaephyte, phanerophyte	Drought adaptation, survival during unfavorable conditions, resistance to disturbance (grazing, fire), adaptation to climate
	Growth-form[1]	categorical	Bulb, erect, graminoid, prostrate, rosette, shrub	Drought strategy, photosynthetic rate, sheltering from severe climatic conditions, resistance to disturbance (e.g. grazing)
	Máx. height[1]	continuous	cm	Dispersal distance, light capture, above-ground competition, resistance to disturbance
Leaf	SLA[3]	continuous	mm^2/mg	Photosynthetic rate and growth, drought adaptation, leaf longevity, decomposition
	Leaf type[1]	categorical	Evergreen, semi-deciduous and green-stemmed leafs; only for woody species	Photosynthetic rate and growth, drought adaptation, nutrient conservation, plant protection, resistance to decomposition

Type	Trait	Type	Categories/units	Function
Reproductive	Onset of flowering[2]	semi-cont.	Initial month	Phenological and reproductive strategy, stress avoidance
	Duration of flowering[2]	semi-cont.	Number of months	
Regenerative	Dispersal strategy[3]	categorical	Anemochory, barochory, ectozoochory, endozoochory	Dispersal ability under spatial and temporal heterogeneity, stability (species pool)
	Seed mass[2]	continuous	mg	Dispersal ability, seedling survival and establishment under unpredictable/harsh conditions, reproductive effort, protection from herbivory, seed persistence
	Seed persistence[2]	ordinal	Transient (<1 year; 0), short-term persistence (1<years<5; 0.5), long-term persistence (> 5 years; 1)	Diversity 'storage', dispersal ability under unpredictable/harsh conditions
Below-ground	Nutrient uptake[2]	binary	Has N-fixing ability or not	Resource acquisition, nutrient cycling
	Máx. root depth[2]	continuous	geometric mean of maximum rooting depth (m) for shrubs, semi-shrubs, perennial grasses, perennial forbs, annuals and succulents in water-limited ecosystems with winter seasonality of precipitation (Schenk and Jackson 2002)	Water and nutrient acquisition, drought adaptation, below-ground competition, soil C sequestration, water fluxes

Table S3.2. Pearson correlation coefficients between single-trait CWM and FDis metrics (N = 54). Significant correlations (P < 0.05) are highlighted in bold.

CWM		FDis											
Trait	Categ.	Lcycle	Lform	Gform	Height	SLA	Ltype	Bflow	Dflow	Dispers	Smass	Slong	Legu	Rdepth
Lcycle		**0.66**												
Lform	Geo		0.09											
	Hemi		**0.40**											
	Ther		**-0.35**											
	Cham		0.19											
	Phan		0.16											
Gform	Erect			**0.50**										
	Gram			0.19										
	Prost			**0.38**										
	Roset			**0.43**										
	Shrub			**-0.52**										
Height					**0.49**									
SLA						**-0.82**								

CWM		FDis												
Trait	Categ.	Lcycle	Lform	Gform	Height	SLA	Ltype	Bflow	Dflow	Dispers	Smass	Slong	Legu	Rdepth
Ltype	ES						**0.41**							
	GS						**0.74**							
	SD						**-0.79**							
Bflow								-0.16						
Dflow									0.25					
Dispers	Anem									**-0.61**				
	Baro									**0.59**				
	Ecto									0.28				
	Endo									0.10				
Smass											-0.07			
Slong												**0.37**		
Legu													**0.99**	
Rdepth														**0.38**

Table S3.3. Pearson correlation coefficients between single-trait FDis metrics (N = 54). Significant correlations (P < 0.05) are highlighted in bold.

FDis	Lcycle	Lform	Gform	Height	SLA	Bflow	Dflow	Dispers	Smass	Slong	Legu	Rdepth
Lcycle	1.00											
Lform	**0.68**	1.00										
Gform	0.13	0.26	1.00									
Height	**0.83**	**0.57**	0.17	1.00								
SLA	**0.82**	**0.43**	-0.10	**0.73**	1.00							
Bflow	-0.18	-0.04	**0.28**	**-0.29**	**-0.36**	1.00						
Dflow	-0.02	0.03	**0.34**	-0.09	-0.26	**0.66**	1.00					
Dispers	**0.74**	**0.47**	**0.38**	**0.67**	**0.64**	0.06	0.06	1.00				
Smass	-0.14	-0.19	0.39	-0.19	**-0.30**	**0.40**	**0.38**	-0.12	1.00			
Slong	**0.66**	**0.52**	-0.11	**0.49**	**0.62**	0.10	0.08	**0.55**	-0.16	1.00		
Legu	0.04	-0.04	-0.11	0.00	0.09	0.12	0.00	0.24	-0.02	**0.39**	1.00	
Rdepth	**0.85**	**0.91**	**0.28**	**0.73**	**0.60**	-0.12	0.05	**0.56**	-0.09	**0.59**	-0.01	1.00

Table S3.4. Weights attributed to each trait in multi-trait FDis calculations (see Table 2): the higher the correlation between single-trait FDis (ρ>0.70), the lower the weight attributed to those correlated traits.

	Trait	weight 13 traits	weight 5 traits	weight HLS
Vegetative	Life cycle	0.2	0.32	
	Life-form	0.1		
	Growth-form	1		
	Máx. height	0.2		0.5
Leaf	SLA	0.2	0.32	0.5

	Trait	weight 13 traits	weight 5 traits	weight HLS
	Leaf type	1		
Reproductive	Onset of flowering	1	1	
	Duration of flowering	1		
Regenerative	Dispersal strategy	0.2	0.36	
	Seed mass	1		1
	Seed persistence	1	1	
Below-ground	N-fixing ability	1		
	Máx. root depth	0.1		

Chapter 4

The effect of inter-annual climatic fluctuations on plant functional traits

Publication in preparation.

4 The effect of inter-annual climatic fluctuations on plant functional traits

4.1 Abstract

Many ecological studies rely on spatial climatic gradients, assuming they mimic changes over time. However, long-term adaptation to local climatic conditions, or fine-scale environmental heterogeneity, may confound the straightforward use of spatial gradients to predict responses to climate over time, although these interactions are seldom considered. Moreover, studies addressing the relative influence of short-term and long-term climatic variations specifically on plant functional traits are lacking. Our aim in this study was to evaluate the effect of inter-annual climatic fluctuations on plant community functional traits, and assess how these fluctuations interact with long-term climate and local topography. To assess this, we studied the functional structure and diversity of plants on Mediterranean Holm-oak woodlands along a spatial climatic gradient, in climatically contrasting years. We considered plant traits related to species strategies to establish, grow, reproduce, disperse and persist under stressful conditions, at the community-level, comprising annual and perennial species. We found that climatic fluctuations between years have an important impact on plant functional traits. However, their effect is modulated by long-term climate and by local topography, which have a stronger influence on trait range and mean, respectively. More limiting climatic conditions, particularly if maintained over longer time spans, led to reduced trait ranges for most of the traits studied. Our findings suggest that functional diversity may show higher resilience than functional structure to short-term climatic fluctuations. Based on this work, we expect more intense and prolonged drought, predicted by climate change models, to have a negative impact on functional diversity of Mediterranean plant communities and associated ecosystem processes.

Keywords: annual plants; community-weighted-mean; functional dispersion; inter-annual; slope

4.2 Introduction

Mediterranean drylands are characterized by hot and dry summers, low precipitation amounts occurring mainly between autumn and spring, and large inter-annual climatic fluctuations (Cody and Mooney, 1978, Davis et al., 1996). Low water availability is the main limiting factor to plants, coupled with very high temperatures in summer, whereas in winter the limiting factors might be more associated with low temperatures (Taylor, 1981, Martínez-Vilalta and Pockman, 2002). According to climate change predictions, in the near future drylands will experience an overall increase in aridity, with simultaneous shifts in both temperature and precipitation regimes (IPCC, 2014). It is therefore essential to understand and anticipate the possible consequences of these climatic changes to dryland plant communities and their associated ecosystem processes. Due to the general lack of information over time, a common approach to study climate effects is the 'space-for-time substitution', which assumes that ecosystems will respond to changing climate over time in the same way that they vary with climate over space (Blois et al., 2013). Such studies along spatial gradients are in most cases based exclusively on perennial species (Gross et al., 2013, Le Bagousse-Pinguet et al., 2016). However, annuals may respond more readily to environmental changes than perennials due to their faster turnover and represent a very important portion of the diversity of dryland plant communities (Noy-Meir, 1973, Aronson et al., 1993).

Despite the basic assumption of 'space for-time substitution', the outcomes of spatial climatic gradients are rarely compared with plant communities' responses to climate over time (but see Aronson and Shmida, 1992, Sala et al., 2012, Cleland et al., 2013). Variation in precipitation between years has an important impact on plant productivity, especially in dry environments (Yang et al., 2008, Miranda et al., 2009, Sala et al., 2012), and are known to affect the composition and diversity of plant communities (Peco et al., 1998, Miranda et al., 2009). Moreover, inter-annual climatic fluctuations may also affect the mean and range of plant functional traits, particularly in communities dominated by annual species (Carmona et al., 2012, Pérez-Camacho et al., 2012, Carmona et al., 2015).

Plant communities are also a result of past climatic circumstances (long-term climate). Legacy-effects of climate on plant communities have been reported on productivity (Schwinning et al., 2004, Sala et al., 2012) and on functional traits (Carmona et al., 2012, Gross et al., 2013, Bagousse-Pinguet et al., 2017, Nunes et al., 2017). These

results have been attributed to the influence of long-term climate on: (i) filtering of species (traits) from an available regional pool; (ii) soil properties (e.g. water storage ability) and nutrient cycling and availability (Delgado-Baquerizo et al., 2013); (iii) biotic legacies, e.g., the effect of previous plant cover and management strategies on nutrient inputs into the soil (Sala et al., 2012); (iv) and seed banks' composition and persistence, which depend on the climatic and biotic history of the site (Peco et al., 2003). Additionally, local environmental conditions, namely topography and soil characteristics, also control moisture availability to plants, by determining water flow paths, accumulation and retention at a local scale, particularly in dry environments (Gómez-Plaza et al., 2001). Hence, interactions between climate and topography (e.g. slope) may affect the functional structure and diversity of dryland plant communities (Le Bagousse-Pinguet et al., 2016).

Previous studies focusing on the effect of inter-annual climatic fluctuations on functional traits of plant communities dominated by annual species, rely on a few functional traits (mostly height, specific leaf area and seed mass), often involve the comparison of only two contrasting years (Carmona et al., 2012, Carmona et al., 2015), or consist of rainfall manipulations at a single study area (Pérez‐Camacho et al., 2012). To our knowledge, none of them considers interactions of inter-annual fluctuations with long-term climate and topography.

More limiting climatic conditions may alter the relative abundance of traits associated to stress-tolerant conservative strategies in relation to stress-avoidant strategies (Ackerly et al., 2002, Gross et al., 2013, Costa-Saura et al., 2016). They may also affect the proportion of plants with different growth-forms (Fay et al., 2002), and affect traits such as plant height (Gross et al., 2013), flowering onset and duration (Kigel et al., 2011, Hänel and Tielbörger, 2015), as well as plant reproductive strategies involving dispersal, seed mass and seed persistence (Baker, 1972, Arroyo et al., 2006, Volis and Bohrer, 2013, Gremer and Venable, 2014). Functional shifts towards the dominance of more favorable functional strategies to cope with stronger climatic constraints, may also reduce the functional dissimilarity between species or trait ranges, following the environmental filtering hypothesis (Grime and Díaz, 2006). It predicts that abiotic filters select species with similar trait values within communities, because species less tolerant to stress would decrease their abundance or be filtered out of the community (Cornwell and Ackerly, 2009).

The aim of this study was to evaluate the effect of inter-annual climatic fluctuations on the mean and range of plant functional traits, and assess how these fluctuations interact with long-term climate and local topography. This was assessed in a plant community of Mediterranean Holm-oak woodlands, which are composed of a sparse tree cover (< 40 trees per ha, on average) (Amaral et al., 1997), and an understory of semi-natural grasslands dominated by annual species, intermingled with shrubland patches. Nine plant traits were considered, including vegetative, reproductive and regenerative traits, related to species strategies to establish, grow, reproduce, disperse and persist under climatically limiting conditions. These traits were studied at the community-level, comprising annual and perennial species. We specifically tested the following hypotheses:

1. More limiting climatic conditions should lead to the dominance of stress-tolerant conservative strategies, and affect the relative abundance of distinct growth-forms, maximum plant height, flowering onset and duration, seed mass and seed longevity;

2. Environmental filtering, due to more limiting climatic conditions, should reduce the ranges of functional traits;

3. Short-term climatic fluctuations should have a major effect on functional traits means and ranges, and interact with climatic history (long-term climate) and topographic characteristics to exert that effect.

4.3 Methods

Study area and environmental variables

The study area is situated in southwestern Iberian Peninsula, Portugal (Alentejo). Climate is Mediterranean, with precipitation occurring mainly between spring and autumn, and hot and dry summers. Mean annual precipitation (50 yrs average) along the study area varies from 522 mm to 617 mm, with a high inter-annual variability (annual precipitation can vary by more than 100 mm between years), and mean annual temperature is ca. 16 ºC. The landscape is characterized by moderate slopes (from 2 to 23º) and dominated by savanna-like Holm-oak woodlands (*Quercus ilex* L. subsp. *rotundifolia*), with a sparse tree cover and variable proportions of semi-natural grasslands

dominated by annual species (ca. 80%), intermingled with shrubland patches. Shrub cover along the studied area ranged from 0% to 55% (average 12%). Soils are dominantly acidic soil (pH<6.5) laying on sedimentary and metamorphic rocks. Field data were collected in 9 sites located along a regional climatic gradient, and each site was sampled in four climatically contrasting years. Sampling sites had moderate to low grazing intensity and no agricultural activities over the last five years. The latter conditions were empirically verified in the field by inspecting evidence of grazing in vegetation, the amount of ungulate pellets, and absence of recent soil tillage.

In dry environments, such as the studied Holm-oak woodlands, inter-annual climatic variability is expected to exert a strong influence on the diversity and productivity of the plant community, especially considering that it is dominated by annual species. However, this influence is likely mediated by the climatic history of the site and also by its topographic and edaphic characteristics (Gómez-Plaza et al., 2001, Schwinning et al., 2004). To assess this, each sampling site was characterized regarding short-term climatic variability between sampling years, and also regarding their long-term climate (Table 4.1; Table S4.1). Short-term climatic data, namely monthly precipitation and maximum and minimum temperatures, were retrieved from local meteorological stations of the Portuguese National Meteorological Institute, IPMA (https://www.ipma.pt/), and spatially modeled using geostatistics. For long-term climate, each site was characterized by a set of 19 climatic variables comprising temperature and precipitation metrics extracted from Worldclim database (www.worldclim.org) with a ~1 km^2 resolution (Hijmans et al., 2005). In addition, to summarize the topographic characteristics of each sampling site, three topographic variables were computed based on digital elevation models with 10 m resolution. Local slope values were used, and also the mean and standard deviation of the slope within a 250 m buffer around the sampling site centroid, to capture local topographic heterogeneity (Table S4.1). Soil characteristics at each site, namely soil organic matter, N content, and C/N ratio, were measured on soil samples collected from the upper 10 cm (composite sample of five subsamples) using standard procedures (Table S4.1).

Table 4.1. Description of the main environmental variables, their Spearman correlations (ρ) with non-metric multidimensional scaling ordination axes (NMDS1 and NMDS2), their range along the study area and respective units. * p<0.05; *** p<0.001 (n=36).

Abbreviation	Description	ρ NMDS1	ρ NMDS2	Range and units
stdev_slope	Standard deviation of the slope within a 250 m buffer around the sampling site centroid (based on digital elevation models, 10 m resolution)	0.62***	0.17	[2.05, 9.92] (º)
slope_250m	Mean of the slope measured within a 250 m buffer around the sampling site centroid (based on digital elevation models, 10 m resolution)	0.79***	0.06	[2.81, 13.71] (º)
LT_precip	Long-term mean annual precipitation	-0.61***	-0.09	[522, 617] (mm)
LT_precip_summer	Long-term precipitation of driest quarter	-0.67***	-0.03	[18, 31] (mm)
Precip_HY	Hydrological year precipitation	-0.15	-0.69***	[280.7, 800.8] (mm)
Tmin_HY	Hydrological year mean of the minimum temperature of the coldest month	0.39*	-0.41*	[-0.9, 7.2] (ºC)
Tmax_HY	Hydrological year mean of the maximum temperature of the warmest month	-0.12	0.32	[29.8, 36.5] (ºC)

Vegetation and functional traits sampling

The plant community was sampled at each site in four climatically contrasting years (Fig. 4.1). Plant sampling was conducted at the peak of standing biomass (April-June) using the point–intercept method (cf. Chapter 2), avoiding drainage lines and flooding surfaces. At each site, species cover was estimated along six 20 m transects systematically arranged over an area of ca. 1000 m^2 and 10 m apart from each other, with points spaced every 50 cm (246 points per site) (for further detail on the sampling scheme see Nunes et al. (2014). At each point, a rod of 5 mm diameter was placed in the ground with a 90° angle. All plant species touching the rod were recorded and the same species was recorded only once at each point. Species cover was calculated as the proportion of points intercepted per transect.

To characterize functional traits of the plant communities, the dominant species attaining ⩾80% of the relative cover within each site were used (Pakeman and Quested, 2007) (108 species overall, and ca. 30 per site). Nine plant traits were selected including continuous, ordinal, and categorical traits, reflecting plant strategies related to establishment, persistence, regeneration, and dispersal (Table 4.2). Trait information was obtained either through direct measurements in the field following standard protocols (Pérez-Harguindeguy et al., 2013), derived from various bibliographic sources, or both (Table 4.2). For each site and sampling year, two functional metrics were calculated for each trait: the community-weighted-mean (CWM) (Garnier et al., 2007) and functional dispersion (FDis) (Laliberté and Legendre, 2010). CWM corresponds to the mean trait value in a community weighted by the relative abundance of the species carrying each value (Garnier et al., 2007). FDis measures the degree of functional dissimilarity within the plant community, and is closely related to Rao's quadratic entropy (Laliberté and Legendre, 2010). It is calculated as the weighted mean distance, in a multidimensional trait space, of individual species from the weighted centroid of all species, where weights correspond to species relative abundances (Laliberté and Legendre, 2010). FDis has several advantages over other indices that measure functional dissimilarity: it takes into account species relative abundances, it is unaffected by species richness, can handle any number and type of traits and is not strongly influenced by outliers (Laliberté and Legendre, 2010). Mean trait values per species were used to compute functional diversity metrics. The Gower distance was used in calculations because it can handle continuous, ordinal, and categorical variables, as well as missing values. All calculations were done with the dbFD function of the FD package (Laliberté et al., 2014) in R (R core Team, 2015).

Statistical Analyses

To assess whether inter-annual climatic variability was driving changes in plant community composition along the sampling sites, a non-metric multidimensional scaling ordination (NMDS) was performed based on species cover at each site, using metaMDS function from vegan package (Oksanen et al., 2013), with Bray-Curtis distance measure. Species cover data was relativized by the maximum of each site (relative abundance) prior to the ordination. Ordination stress statistic was used as a measure of goodness of fit. Then, a permutational multivariate analysis of variance (PERMANOVA) was performed with 9999 permutations on Bray-Curtis communities' dissimilarities, to test for

the effect of 'Year' (Oksanen, 2011). Afterwards, the significance of correlations between NMDS ordination axes and the environmental variables initially considered was tested, comprising short and long-term climatic variables, as well as topographic and edaphic characteristics (Table S4.1). Environmental variables were superimposed on the NMDS ordination, and the significance of correlations was determined by means of Spearman correlations, to account for possible nonlinear relationships (Table S4.1).

To assess the degree of inter-annual climatic variability, the effect of 'Year' (coded as a factor) on short-term climatic variables (precipitation, minimum and maximum temperature) was tested by means of mixed effects models, with site as a random factor, using the R package nlme (Pinheiro et al., 2017). Afterwards, Tukey pairwise comparisons with Bonferroni corrections were performed to check specific differences between years. Secondly, after exploring the correlation among the climatic, topographic and edaphic variables at each site, and because many of them were strongly correlated, they were summarized into 5 variables, the ones more strongly correlated with NMDS axes (Table S4.1) and showing a Pearson´s r correlation <0.70 between them (Table S4.2), to be used in subsequent analysis. In addition, 'Time' was considered as an additional continuous predictor, representing the natural successional progression of the plant community. In short, the predictors used in modelling procedures described below were: cumulative precipitation of the hydrological year (from September to August), minimum temperature of the coldest month, maximum temperature of the warmest month, long-term annual precipitation (mean of 50 years), slope mean within a 250 m buffer around the sampling site centroid (Table 4.1), and time. Furthermore, we also included interactions among these predictors in full models, because long-term climate and topo-edaphic characteristics can largely modulate the effect of short-term climate e.g. on water availability for the plant community.

The influence of all predictors on single-trait CWM and FDis was then examined for the nine traits studied, by means of mixed effects models, with site as a random factor, using the R package nlme (Pinheiro et al., 2017). Response variables were log or square-root transformed prior to analysis, to meet linear models' assumptions. All predictors were standardized before analyses using the Z-score to interpret parameter estimates on a comparable scale. To select the best predictors for each response variable, a model selection procedure based on two criteria was used. Model simplification was performed by using a backward selection, removing non-significant predictors that did not impact model predictive ability (R^2), and simultaneously minimizing

the Akaike information criterion (AIC). First, all models only with significant predictors and with lower AIC were kept. Then, the best predictors most supported by the data were selected, relying simultaneously on ecological coherence and on the information of predictor's relevance provided by the function dredge in the R package MuMIn (Barton 2016). During the selection of the fixed effects model fitting was done using Maximum Likelihood estimation (ML), and turned to Restricted Maximum Likelihood (REML) once the final model was selected (Zuur et. al, 2009). Model residuals were graphically inspected for constant variance and normality. The goodness-of-fit of the final models was evaluared by means of the marginal R^2 (i.e. variance explained by fixed factors) and conditional R^2 (i.e. variance explained by fixed and random factors) (Nakagawa and Schielzeth, 2013). To evaluate the relative effect of each predictor on functional metrics calculated for the nine traits studied, an approach similar to the variance decomposition analysis based on predictors' Z-scores was used. Since predictors were standardized before analyses, the relative effect of each predictor can be calculated as the ratio between its standardized parameter estimate and the sum of all parameter estimates. This ratio was used to calculate the relative effect of each predictor (expressed as % of variance explained) in relation to the marginal R^2 of the corresponding model. Finally, because local variations in slope were found to have, in interaction with short-term climate, a determinant influence on plant community structure, the effect of 'Year' (coded as factor) on the CWM of shrub growth-form was further tested separately for sites with low slope and with high slope. This was achieved by means of mixed effects models, with site as a random factor, followed by Tukey pairwise comparisons with Bonferroni corrections, to check specific differences between years in both circumstances. All the analysis were performed under R statistical environment (R coreTeam, 2015).

4.4 Results

The climate during the four sampling years varied considerably (Fig. 4.1). The year 2011 was the wettest (mean precipitation ca. 700 mm), while 2012 was the driest year (mean precipitation ca. 340 mm) and also the one with lower temperatures during winter (Fig. 4.1, Table S4.3). While 2011 and 2014 showed maximum and minimum temperatures close to the long-term mean, 2012 and 2015 showed more extreme

temperatures along the year, and 2015 showed the highest maximum temperature of the warmest month (Fig. 4.1, Table S4.3).

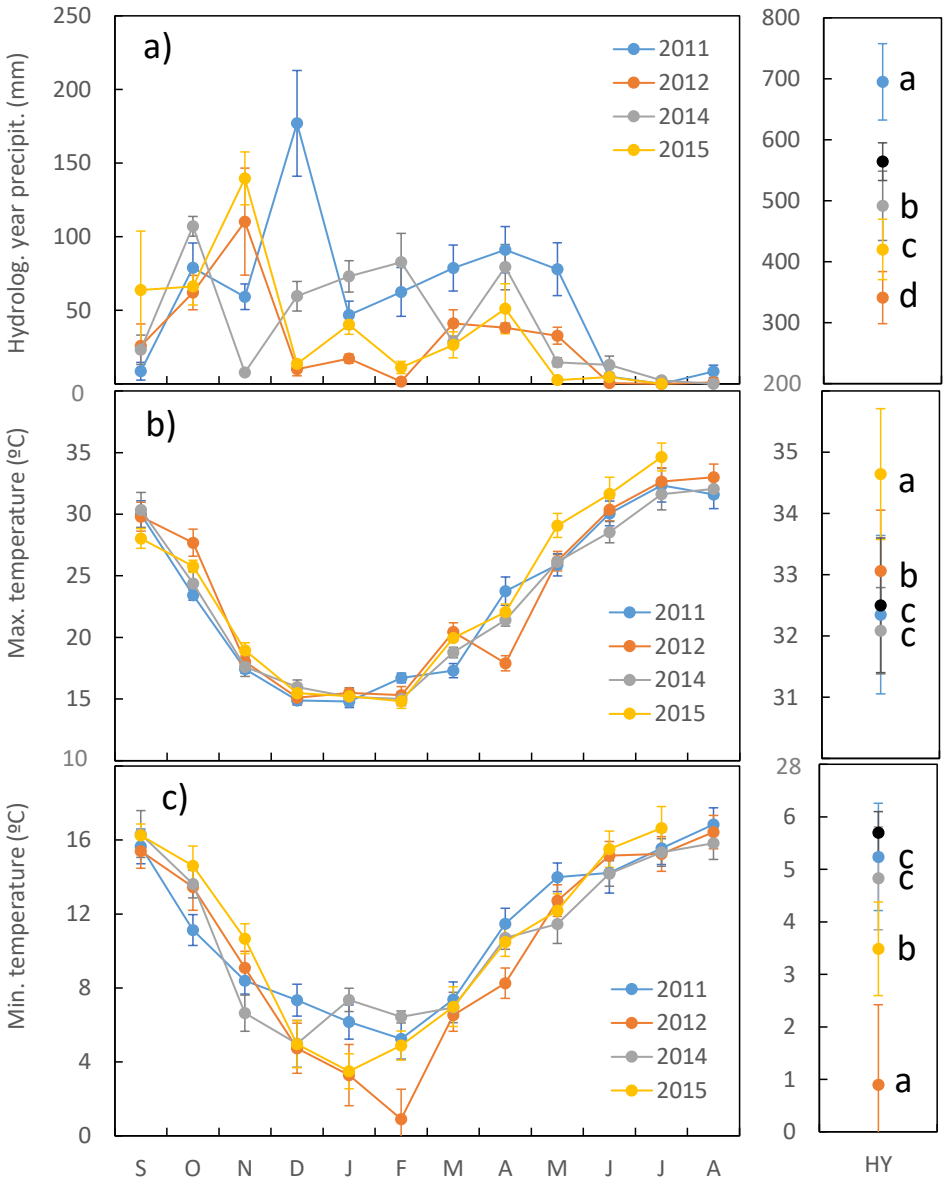

Figure 4.1. Variation of climatic variables during the hydrological year (from September to August) for the four years of the study: a) monthly precipitation (left) and annual mean for each year (right); b) monthly maximum temperature (left) and mean of the maximum temperature of the warmest month for each year (right); c) monthly minimum temperature (left) and mean of the minimum temperature of the coldest month for each year (right). Black dots represent long-term values (average of 50 years). (mean ± SD, n=9 sites). Values displaying different lowercase letters have significantly different means between years (Table S4.3).

The main gradients in species composition were described by a 2-dimensional NMDS ordination with a final stress value of 0.17 (Fig. 4.2). The first axis (NMDS1 hereafter) explained 40.2% of the variation in species ordination. It was strongly correlated with variables characterizing slope variations within each sampling site, and with long-term climate (Fig. 4.2, Table 4.1). The second axis (NMDS2 hereafter) explained 10.7% of the variation in species composition and showed stronger correlations with short-term climate (Fig. 4.2, Table 4.1). Plant community composition changed significantly between years (PERMANOVA test: F=2.02, p-value=0.041).

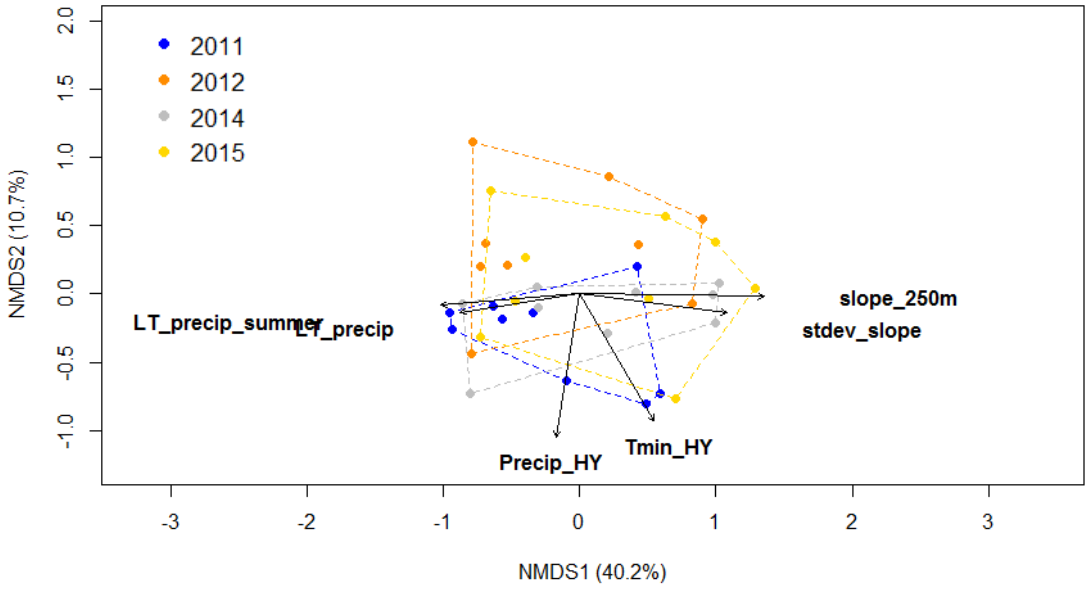

Figure 4.2. Non-metric multidimensional scaling (NMDS) based on species cover, with points representing sites sampled in different years (2011, 2012, 2014, 2015), and vectors representing the environmental variables more strongly correlated with NMDS axes (see Table 4.1 and Table S4.1 for more information). Final stress for the 2-dimensional configuration was 0.17.

On average, the plant community was dominated by short-lived species, mainly by graminoids (ca. 53%) and species with anemochorous dispersal (ca. 80%) (Table 4.2). Specific leaf area (SLA) showed the highest mean value of FDis among all traits (Table 4.2).

Table 4.2. Description of the plant functional traits studied, and their community-weighted-mean (CWM) and functional dispersion (FDis) (mean ± SD). The sources used to obtain trait information are indicated by superscript numbers: [1]directly observed or measured in the field following standard protocols (Pérez-Harguindeguy, et al. 2013); [2]derived from various bibliographic sources (see Appendix S1); [3]obtained both through direct measurement in the field and derived from bibliographic sources. (n=36; 9 sites x 4 years). SLA – specific leaf area.

Trait	Type	Categories*/units*	CWM	FDis
Vegetative				
Life cycle[3]	Ordinal	Annual (0), biennial (0.5) or perennial (1)	0.15 ± 0.17	0.51 ± 0.44
Growth-form[1]	Categorical	Bulb	0.01 ± 0.04	0.29 ± 0.05
		Erect	0.10 ± 0.07	
		Graminoid	0.53 ± 0.14	
		Prostrate	0.04 ± 0.05	
		Rosette	0.21 ± 0.14	
		Shrub	0.12 ± 0.16	
Max. height[1]	Continuous	*cm*	29.34 ± 12.25	0.50 ± 0.38
Leaf				
SLA[3]	Continuous	mm^2/mg	24.91 ± 3.93	0.83 ± 0.14
Reproductive				
Onset flower.[2]	Semi-quant.	*initial month*	3.26 ± 0.25	0.49 ± 0.19
Duration flower.[2]	Semi-quant.	*nr. of months*	4.50 ± 0.45	0.54 ± 0.25
Regenerative				
Dispersal strategy[3]	Categorical	Anemochory	0.81 ± 0.15	0.19 ± 0.09
		Barochory	0.16 ± 0.15	
		Ectozoochory	0.02 ± 0.02	
		Endozoochory	0.01 ± 0.02	
Seed mass[2]	Continuous	*mg*	0.98 ± 0.44	0.35 ± 0.20
Seed persistence[2]	Ordinal	Transient (<1 year; 0), short-term persistence (1<years<5; 0.5), long-term persistence (> 5 years; 1)	0.49 ± 0.09	0.38 ± 0.27

The passage of time, higher slope and higher minimum temperatures, and their interactions, favored species with longer life-cycles, mostly shrubs with lower SLA (Fig.

4.3, Fig. 4.4). The proportion of erect and prostrate growth-forms in the community was lower under higher maximum temperatures, while graminoids decreased their relative cover with higher minimum temperatures during the hydrological year, although the respective models explained a low proportion of the variation in these functional groups ($r2 \leqslant 0.20$) (Fig. 4.3). The relative cover of rosettes was favored by higher short-term precipitation, showing the opposite trend with higher minimum temperatures during the hydrological year and with higher long-term precipitation (Fig. 4.3). The mean height of the plant community was explained by short-term climatic variables and their interactions with slope. It decreased with higher short-term precipitation and increased with higher minimum temperature (Fig. 4.3). Onset of flowering occurred latter with higher long-term precipitation, which interacted with slope, whereas community flowering duration decreased with higher maximum temperatures during the hydrological year (although it explained a low proportion of its variation, $R^2 < 0.20$) (Fig. 4.3). Higher precipitation and lower minimum temperature during the hydrological year led to a relative increase in anemochorous dispersal at the expense of barochory, which showed the opposite trend (Fig. 4.3). Ecto- and endozoochory dispersal were uncommon in the plant community ($\leqslant$ 2%) and their variation was not explained by any of the environmental variables considered. The predictors considered explained a small proportion of the variation in community seed mass mean ($R^2 = 0.21$). Seed persistence of the sampled community decreased with higher precipitation and increased with higher minimum temperatures during the hydrological year, being also influenced by slope and its interaction with short-term climate (Fig. 4.3).

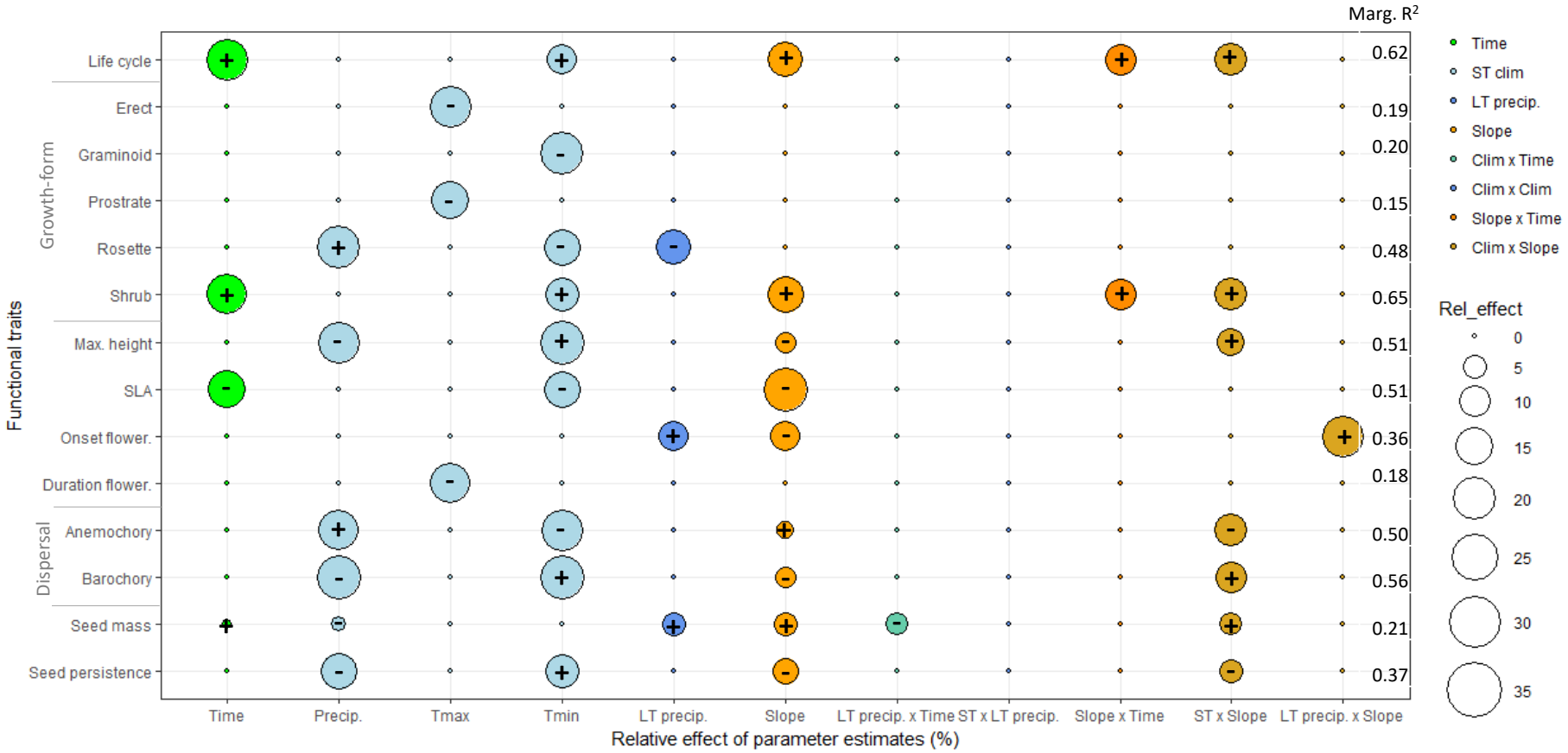

Figure 4.3. Relative effect of parameter estimates of model predictors and their interactions on the community-weighted means of the nine traits studied (except bulb growth-form and ecto and endozoochory dispersal strategies, for which models were not significant). The area of the circles, designated by 'relative effect', expresses the relative importance of each factor as the percentage of explained variance. Marginal R^2 of each model is displayed on the right. Each color represents a type of predictor: time (green); short-term climate (light blue); long-term precipitation (dark blue) and slope (orange), and their interactions (see legend on the right). Signs of the standardized coefficients for each predictor are displayed inside the circles (see Table S4.4 and S4.7 for further analyses details). ST - short-term climate; LT precip. – Long-term precipitation.

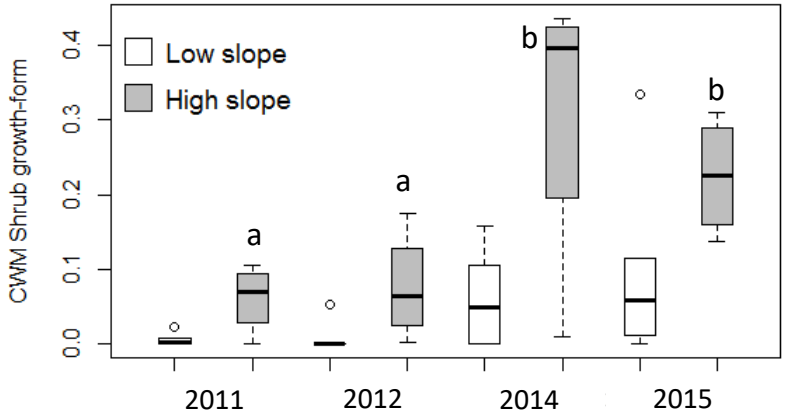

Figure 4.4. Variation of community-weighted-means (CWM) for the shrub growth-form along the sampled years for sites with high slope (>7º; n=4) and sites with low slope (<7º; n=5). Values displaying different lowercase letters have significantly different means (see Table S4.5 for analyses details).

Time had a positive and relevant effect on FDis for most of the traits, except for flowering traits (Fig. 4.5). The functional dispersion of life-cycle was favored by time and slope, and was also positively influenced by long-term precipitation, and negatively by its interaction with time (Fig. 4.5). The diversity of growth-forms was negatively influenced by higher maximum temperatures, and less strongly by higher long-term precipitation, as well as by its interaction with short-term climate and time (Fig. 4.5). The plant community showed a higher dispersion in maximum height with time, lower maximum and higher minimum temperatures and slope, being also influenced by interactions among them and with long-term precipitation (Fig. 4.5). The diversity in SLA values was favored over time, and by higher minimum temperatures and higher long-term precipitation, and by its interaction with slope (Fig. 4.5). A higher diversity in flowering onset was favored by lower maximum temperature and higher long-term precipitation, and also positively influenced by interactions among them and with slope, which by itself had the opposite effect (lower FDis) (Fig. 4.5). The functional diversity of flowering duration was negatively influenced by higher maximum and higher minimum temperatures, although the corresponding model showed a relatively low explanation power (r2 = 0.25) (Fig. 4.5). Dispersal strategies showed a lower functional dispersion under higher maximum temperatures, and were negatively affected by long-term climate and its interaction with time, although with a weaker relative effect (Fig. 4.5). The variability in community seed mass was favored by short and long-term precipitation and by lower maximum temperatures (lower stress), and negatively by their interaction. The dispersion of seed persistence was positively influence by higher minimum temperatures and by a higher slope and their interaction (Fig. 4.5).

Figure 4.5. Relative effect of parameter estimates of model predictors and their interactions on the functional dispersion of the nine traits studied. The area of the circles, designated by 'relative effect', expresses the relative importance of each factor as the percentage of explained variance. Marginal R^2 of each model is displayed on the right. Each color represents a type of predictor: time (green); short-term climate (light blue); long-term precipitation (dark blue) and slope (orange), and their interactions (see legend on the right). Signs of the standardized coefficients for each predictor are displayed inside the circles (see Table S4.6 and S4.8 for further analyses details). ST - short-term climate; LT precip. – Long-term precipitation.

Overall, although the climatic conditions during the sampling year, i.e. short-term climate, did influence the functional metrics for most of the traits studied, they were more important in explaining CWM than FDis values (Fig. 4.6). The same happened for slope and its interaction with other predictors. Conversely, long-term precipitation and its interaction with other environmental variables, and also time, had a higher relative effect in FDis, in comparison with CWM (Fig. 4.6).

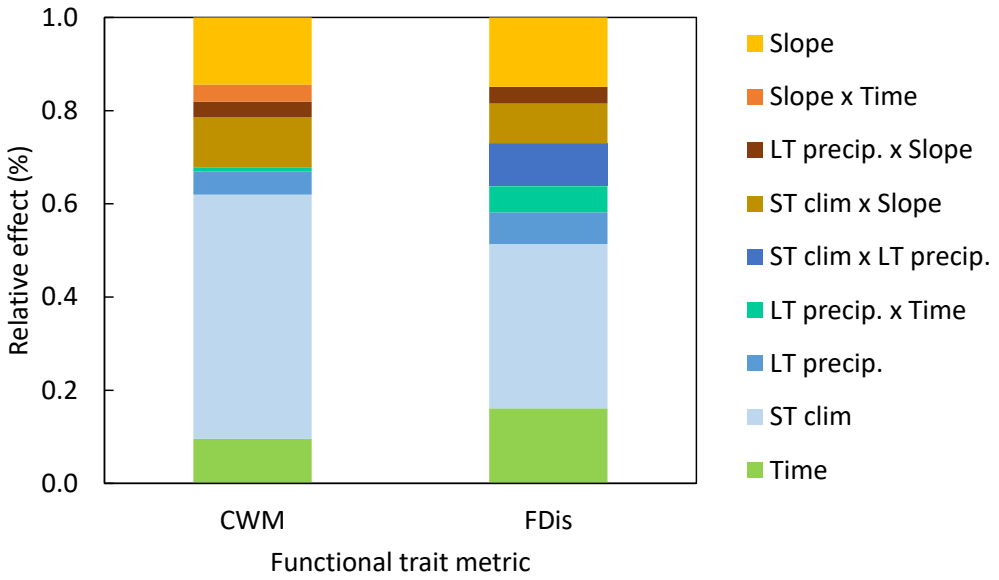

Figure 4.6. Sum of relative effect of each predictor for single-trait CWM and FDis metrics of all traits.ST clim – short-term climate; LT precip. – Long-term precipitation.

4.5 Discussion

The results show that inter-annual climatic fluctuations, characteristic of Mediterranean drylands, have a considerable effect on the means and ranges of plant functional traits. Moreover, their effect is modulated by local topographic characteristics and by past climate. The relative effect of these environmental factors was also found to depend on the functional metric considered: short-term climate and topography have a larger relative effect on the community trait means (CWM), while long-term climate has a more important role shaping trait functional dispersion.

Changes in CWM in response to environmental limiting factors

The increase in shrub cover was associated to an increase in life-cycle and a decrease in SLA (i.e. more conservative strategies) was largely determined by

successional dynamics (time) and increased slope, instead of climatic stress (water and temperature limitations), contrary to hypothesis 1. This may be due to the control that topographic characteristics exert on the distribution of water along the soil profile, particularly in dry environments (Gómez-Plaza et al., 2001). On hillslopes runoff and erosion following rainfall events tend to promote water storage at lower depths thus favoring shrubs, whereas in plain areas moisture is retained at upper soil layers promoting the dominance of herbaceous species (Sala et al., 1997, McAuliffe, 2003). In addition, in Mediterranean Holm-oak woodlands an increase in shrub dominance is expected to occur as the result of natural successional dynamics, when agricultural activities or grazing decrease in intensity or cease (Castro and Freitas, 2009). This may also contribute to higher shrub cover on more hilly areas, where grazing is usually less intense (Bailey et al., 1996) and agricultural activities are more difficult to perform. Regardless of the effect of these factors, a lower minimum temperature promoted shorter life-cycle and higher SLA, towards a higher relative abundance of biannual and annual species. Previous studies found an increase in the abundance of short-lived stress-avoidant species with high SLA under severe arid conditions (Ackerly et al., 2002, Gross et al., 2013), which often undergo climatic extremes. In addition, there are previous reports of low minimum temperatures affecting shrub survival in arid environments (Martínez-Vilalta and Pockman, 2002). The increase in the relative abundance of graminoid species under more limiting conditions followed our hypothesis, and might have also contributed to the increase of SLA in the community, as they have usually higher SLA compared to other herbaceous growth-forms. The proportion of rosettes also increased under such circumstances (lower minimum temperature and lower long-term precipitation) in accordance with previous studies (Nunes et al., 2017), although it increased with precipitation during the hydrological year.

Increased height was found with higher minimum temperature, but lower height under higher short-term precipitation, partially confirming hypothesis 1. This may be because higher precipitation during the hydrological year may favour, at least in plain areas, the dominance of herbaceous species (Sala et al., 1997, McAuliffe, 2003), which are shorter than shrubs. The observed positive effect of the interaction between slope and short-term climate on mean height, supports this assumption to some extent.

As expected, the onset of flowering occurred latter (higher CWM) under more favorable climatic conditions. Interestingly, its CWM showed a stronger dependence on long-term than on short-term precipitation. This trait is expected to be more dependent

on the identity of the species present in the community, which are the result of a climatic filtering operating over the long-term on the available regional pool. Furthermore, in Mediterranean woodlands long-term precipitation is a strong determinant of phenological events, having a positive effect on the temporal extent of plant growing season (Ramos et al., 2015). Accordingly, a shorter flowering duration was found under higher stress (higher maximum temperature), despite the low explanation power of the corresponding model. The CWM of dispersal strategies showed no clear pattern is response to higher stress, since short-term precipitation promoted anemochory, while higher minimum temperature favored barochory. Environmental filtering in this case, may promote the coexistence of different strategies with multiple co-optimal combinations as a way to increase the odds of successful dispersion under severe climatic conditions (Venable and Brown, 1988, Volis and Bohrer, 2013). The same seemed to happen with seed mass CWM, as short and long-term precipitation showed negative and positive effects, respectively, although the former exhibited a weaker explanation power than the latter. Seed persistence is expected to increase under harsher environments, and our results confirmed this trend: higher short-term precipitation had a negative effect on the community seed persistence mean. However, this trait appears to be sensitive to low temperatures, increasing its mean with higher minimum temperature. Low Autumn temperatures are thought to influence dormancy breaking of hard seeds, particularly of leguminous species (Taylor, 1981), and low temperatures may limit the germination of seeds with longer persistence.

Stronger climatic limitations led to decreased functional dispersion for most traits

In accordance to hypothesis 2, the results suggest that species with traits that confer a lower tolerance to the main climatic limiting factors may decrease in abundance or be filtered out of the community. Conversely, under more favorable conditions, competitive interactions may be stronger and thus favor the coexistence of a higher diversity of functional strategies. This is in accordance with previous studies, which found lower functional diversity for different sets of plant traits under drier conditions in Mediterranean grasslands (Cornwell and Ackerly, 2009, Carmona et al., 2012, Nunes et al., 2017). Life-cycle, height, SLA, onset of flowering, seed mass and seed persistence showed an unequivocal trend towards lower FDis under more limiting climatic conditions. Growth-form and dispersal strategies, on the other hand, exhibited somewhat contradictory results. They showed lower FDis under higher short-term climatic constraints (higher maximum temperature and/or lower minimum temperature), but

displayed the opposite trend with long-term precipitation, which had a negative effect, although weaker, on FDis. This suggests that these traits respond promptly and more strongly to climatic fluctuations between years, being less influenced by past climatic history than the other traits studied. As such, climatically contrasting consecutive years may cause rapid changes in the relative abundance, for example, of graminoid *versus* erect growth-forms, or of anemochorous *versus* barochorous dispersal, respectively. Flowering duration FDis showed no clear pattern under more limiting climatic conditions.

Time course had a positive, and more prominent effect on FDis for most of the traits, than on CWM, despite the considerable climatic variability among consecutive years, i.e. alternation between wet and dry years. The course of time represents the natural successional dynamics in these communities, which generally imply a change from the dominance of herbaceous species to shrub-dominated communities (Castro and Freitas, 2009). At the intermediate phase of this transition, it is reasonable to admit the co-existence of plants with contrasting strategies and thus a higher functional dispersion. As the successional transition progresses towards a new dominance, we would expect FDis to decrease again, depicting a unimodal behavior along the successional process.

Relative effect of short and long-term climate on trait metrics

The results confirm the hypothesis that short-term climate would affect the mean and range of most functional traits studied. However, the availability of water inputs from precipitation to the plant community depends on topographic control of water flows (Gómez-Plaza et al., 2001). This interaction between climate and topography was clearly demonstrated by the considerable relative effect of slope variations especially on the functional structure (CWM) of the studied plant communities, particularly for life-cycle, shrub growth-form and SLA traits.

It is reasonable to assume that the functional pattern of plant communities, being influenced by short-term climatic variations, had already been shaped by long-term past climate. Previous studies reported legacy-effects of climate, e.g. on productivity (Schwinning et al., 2004, Sala et al., 2012) , but also on functional traits (Carmona et al., 2012, Nunes et al., 2017). This legacy-effect is likely associated to climatic filtering of traits from an available regional pool over time, and to the effect of long-term climate on soil properties and nutrient availability (Delgado-Baquerizo et al., 2013), and on seed banks' composition and persistence (Peco et al., 2003). The present results support this

legacy-effect, as long-term climate exerted a significant relative effect on plant trait metrics, particularly on FDis. The stronger relative effect of long-term precipitation on FDis, when compared to CWM, might be because functional dispersion, as a 'diversity' metric, depends more on the array of species (traits) that make up the community, while CWM essentially reflects the traits of the dominant species. Hence, FDis might be more influenced by the climatic filtering acting on the trait regional pool over the long-term. As a consequence, FDis may show higher resilience than CWM to short-term climatic fluctuations, as suggested in previous studies (Carmona et al., 2012), although this may depend on the particular trait being addressed (Carmona et al., 2015).

It is important to notice that, by using a single trait value per species regardless of the year of sampling, this study only considers alterations in functional metrics due to changes in species composition and abundance between years. Nevertheless, some of the traits studied may show considerable intraspecific variability in response to climatic short-term variations (e.g. height). Therefore, it would be important to consider intraspecific variability in future studies addressing shifts in plant functional traits associated with inter-annual climatic variability, in order to ascertain its relevance.

Concluding remarks

Previous studies addressing the effect of inter-annual climatic fluctuations on plant traits of communities dominated by annual species rely on few traits (<4), often involve the comparison of no more than two contrasting years, and lack the evaluation of the relative effect of long-term climate on inter-annual functional variations. By comparing the community mean (CWM) and dissimilarity (FDis) of plant functional traits in four climatically contrasting years comprising nine plant traits, it was possible to advance the knowledge gathered so far and provide a clearer picture on the relative effect of short and long-term climate and of topography on functional trait metrics. The present findings demonstrate that climatic fluctuations between years have an important impact on plant functional trait metrics, and therefore imply that this temporal variability should be taken into account in studies involving sampling at a single year. The results also show that the effect of short-term climate on community functional traits is modulated both by the local topography, and the climatic past, which have a stronger influence on CWM and FDis, respectively. Hence, these findings suggest that FDis may show higher resilience than CWM to short-term climatic fluctuations. Still, more limiting climatic conditions, particularly if maintained over longer time spans, led to a reduction

of functional dispersion for most of the traits studied. This indicates that drier conditions foreseen by climate change predictions may negatively impact functional diversity of Mediterranean plant communities and associated ecosystem processes.

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Supporting Information

Appendix S1. List of the bibliographic sources used for trait data.

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Table S4.1. Description and range of the environmental variables considered, and their Spearman correlations (ρ) with non-metric multidimensional scaling (based on species cover) ordination axes NMDS1 and NMDS2. The variables retained as predictors in mixed effect models are highlighted in bold. * p<0.05; ** p <0.01; *** p <0.001.

Environmental variables	Min.	Max.	ρ NMDS1	ρ NMDS2
TOPOGRAPHIC				
Slope (at the centroid of the sampling point)	2.4	22.9	-0.06	0.08
Stdv_slope (standard deviation of the slope within a 250 m buffer around the sampling point, based on digital elevation models, 10 m resolution)	2.0	9.9	0.62***	0.17
Slope_250m (standard deviation of the slope within a 250 m buffer around the sampling point, based on digital elevation models, 10 m resolution)	2.8	13.7	**0.79*** **	0.06
SHORT-TERM CLIMATE				
Precip. previous hydrol. year (mm)	427.1	1068.2	-0.22	-0.36*
Precip. previous civil year (mm)	453.8	1009.2	-0.28	-0.49**
Precip. previous Autumn (Sep-Nov, mm)	113.6	344.8	0.02	0.16
Precip. current civil year (mm)	435.2	727.1	-0.15	-0.69***
Precip. current hydrol. year (mm)	280.7	800.8	-0.05	**-0.76*** **
Hydrol. year mean of the min. temperature of the coldest month	-0.9	7.2	0.39*	**-0.41***
Hydrol. year mean of the max. temperature of the warmest month	29.8	36.5	-0.12	0.32
LONG-TERM CLIMATE				
Annual mean temperature	16.1	16.9	-0.05	-0.19
Mean diurnal range (Mean of monthly (max temp - min temp))	9.5	10.9	-0.35*	0.25
Isothermality (* 100)	40	42	0.09	0.04
Temperature Seasonality (standard deviation *100)	4348	5478	-0.42*	0.04
Max temperature of warmest month	28.8	32.5	-0.43**	0.02
Min Temperature of coldest Month	5.7	6.9	-0.13	-0.34*
Temperature annual range	22.2	26.8	-0.35*	0.2
Mean temperature of wettest quarter	10.6	12	0.32	-0.24
Mean temperature of driest quarter	21.8	23.9	-0.42*	0.02
Mean temperature of warmest quarter	22.2	24.2	-0.42*	0.02
Mean temperature of coldest quarter	10.2	11.2	0.21	-0.26
Annual precipitation	522	617	**-0.61*** **	-0.09
Precipitation of wettest month	73	91	0.22	-0.27
Precipitation of driest month	1	3	-0.55***	-0.03
Precipitation seasonality (coef. of variation)	57	68	0.53***	0.06
Precipitation of wettest quarter	213	262	0.12	-0.29
Precipitation of driest quarter	18	31	-0.67***	-0.03
Precipitation of warmest quarter	20	31	-0.49**	0.02

Environmental variables	Min.	Max.	ρ NMDS1	ρ NMDS2
Precipitation of coldest quarter	204	258	-0.31	-0.29
EDAPHIC				
Soil organic matter (year 2012, %)	2.34	10.14	0.71***	-0.08
Soil N content (year 2012, %)	0.09	0.17	0.72***	-0.14
Soil C/N (year 2012)	7.46	12.53	-0.37*	-0.07

Table S4.2. Pearson correlations between the variables retained as predictors in mixed effect models. * p<0.05; ** p <0.01; *** p <0.001.

	Precip_HY	Tmin_HY	Tmax_HY	LT precip.
Precip _HY				
Tmin_HY	0.64***			
Tmax_HY	-0.42*	-0.41*		
LT precip.	0.17	-0.27	-0.21	
Slope	-0.11	0.40*	-0.19	-0.47**

Table S4.3. Results of the mixed effect models fitted to short-term climatic variables of the hydrological year (Sep-Aug), using 'Year' as the predictor factor, and respective Tukey multiple comparisons with Bonferroni corrections. Response variables: cumulative precipitation of the current hydrological year (Precip. HY), mean of the minimum temperature of the coldest month (Tmin HY), mean of the maximum temperature of the warmest month (Tmax HY).

Response variable	df	F-value	p-value	Null hypothesis	Estimate	Std error	z-value	p-value
Precip. HY	24	199.47	<0.0001	2012-2011==0	-0.716	0.039	-18.32	<0.001
				2014-2011==0	-0.348	0.039	-8.91	<0.001
				2015-2011==0	-0.507	0.039	-12.97	<0.001
				2014-2012==0	0.368	0.039	9.41	<0.001
				2015-2012==0	0.209	0.039	5.35	<0.001
				2015-2014==0	-0.158	0.039	-4.06	<0.001
Tmin HY	24	87.8	<0.0001	2012-2011==0	-4.337	0.296	-14.68	<0.001
				2014-2011==0	-0.406	0.296	-1.37	1
				2015-2011==0	-1.749	0.296	-5.92	<0.001
				2014-2012==0	3.932	0.296	13.31	<0.001
				2015-2012==0	2.588	0.296	8.76	<0.001
				2015-2014==0	-1.343	0.296	-4.55	<0.001
Tmax	24	79.41	<0.0001	2012-2011==0	0.714	0.182	3.92	<0.001
				2014-2011==0	-0.262	0.182	-1.44	0.905
				2015-2011==0	2.295	0.182	12.59	<0.003
				2014-2012==0	-0.976	0.182	-5.35	<0.004
				2015-2012==0	1.582	0.182	8.67	<0.005
				2015-2014==0	2.557	0.182	14.03	<0.006

Table S4.4. Results of the mixed effect models fitted to single-trait community-weighted-means metrics after backward selection, and model choice based on the lowest AIC.

	Traits/Func. groups	predictor	Std.coef	Std_error	t-test p-value	df	Rel. effect (%)	Marg. R^2
Growth-form	Life cycle	Tmin_HY	0.0339	0.0145	0.0283	23	8.84	0.62
		slope	0.0517	0.0194	0.0321	7	13.46	
		Time	0.0732	0.0136	0.0000	23	19.06	
		Tmin_HY:slope	0.0419	0.0142	0.0071	23	10.93	
		slope:Time	0.0383	0.0136	0.0096	23	9.98	
	Bulb	*ns*						
	Erect	Tmax_HY	-0.0543	0.0186	0.0071	26	18.99	0.19
	Graminoid	Tmin_HY	-0.0440	0.0142	0.0047	26	19.54	0.20
	Prostrate	Tmax_HY	-0.0523	0.0172	0.0054	26	14.77	0.15
	Rosette	Precip_HY	0.1512	0.0283	0.0000	25	20.58	0.48
		Tmin_HY	-0.1053	0.0298	0.0016	25	14.33	
		LTprecip	-0.0988	0.0267	0.0076	7	13.45	
	Shrub	Tmin_HY	0.0440	0.0143	0.0053	23	12.12	0.65
		slope	0.0514	0.0169	0.0189	7	14.14	
		Time	0.0649	0.0127	0.0000	23	17.85	
		Tmin_HY:slope	0.0396	0.0146	0.0126	23	10.88	
		slope:Time	0.0370	0.0129	0.0086	23	10.18	
	Max. height	Tmin_HY	0.3500	0.0694	0.0000	24	21.93	0.51
		slope	-0.0585	0.0629	0.3835	7	3.67	
		Precip_HY	-0.2933	0.0628	0.0001	24	18.38	
		Tmin_HY:slope	0.1173	0.0452	0.0159	24	7.35	
	SLA	Tmin_HY	-0.0561	0.0213	0.0142	25	14.44	0.51
		Time	-0.0587	0.0190	0.0048	25	15.10	
		slope	-0.0817	0.0281	0.0227	7	21.02	
	Onset flower.	slope	-0.1123	0.0429	0.0472	5	8.57	0.36
		LTprecip	0.1167	0.0635	0.1255	5	8.90	
		slope:LTprecip	0.2451	0.0856	0.0352	5	18.70	
	Duration flower.	Tmax_HY	-0.0421	0.0153	0.0106	26	17.80	0.18
Dispersal strategy	Anemochory	Precip_HY	0.0675	0.0166	0.0004	24	17.37	0.50
		Tmin_HY	-0.0750	0.0182	0.0004	24	19.31	
		slope	0.0096	0.0141	0.5183	7	2.48	
		Tmin_HY:slope	-0.0416	0.0122	0.0024	24	10.70	
	Barochory	Precip_HY	-0.1532	0.0290	0.0000	24	20.99	0.56
		Tmin_HY	0.1522	0.0319	0.0001	24	20.86	
		slope	-0.0288	0.0267	0.3172	7	3.94	
		Tmin_HY:slope	0.0737	0.0211	0.0019	24	10.09	
	Ectozoochory	*ns*						

Traits/Func. groups	predictor	Std.coef	Std_error	t-test p-value	df	Rel. effect (%)	Marg. R^2
Endozoochory	*ns*						
Seed mass	Precip_HY	-0.0237	0.0252	0.3571	23	1.45	0.21
	slope	0.0871	0.0768	0.3003	6	5.33	
	Time	0.0079	0.0242	0.7487	23	0.48	
	LTprecip_	0.0807	0.0770	0.3349	6	4.94	
	Precip_HY:slope	0.0691	0.0191	0.0014	23	4.23	
	Time:LTprecip	-0.0697	0.0187	0.0011	23	4.26	
Seed persistence	Precip_HY	-0.0450	0.0121	0.0011	24	13.47	0.37
	slope	-0.0208	0.0101	0.0792	7	6.23	
	Tmin_HY	0.0403	0.0131	0.0052	24	12.07	
	Precip_HY:slope	-0.0165	0.0080	0.0484	24	4.96	
N-fixing ability	*ns*						

Table S4.5. Results of the mixed effect models fitted to community-weighted-means for shrub growth-form, using 'Year x Slope' as predictors, and respective Tukey multiple comparisons with Bonferroni corrections. Sampling sites were divided into two groups: high slope (>7; n=4 x 4 years) and low slope (<7; n=5 x 4 years), and the analyses were done separately for each group.

Response variable	df	F-value	p-value	Null hypothesis	Estimate	Std error	z-value	p-value
low slope (<7) n=20	12	2.436	0.1	2012-2011==0	0.004149	0.041802	0.099	1
				2014-2011==0	0.055864	0.041802	1.336	1
				2015-2011==0	0.097017	0.041802	2.321	0.122
				2014-2012==0	0.051715	0.041802	1.237	1
				2015-2012==0	0.092868	0.041802	2.222	0.158
				2015-2014==0	0.041153	0.041802	0.984	1
high slope (>7) n=16	9	9.015	0.0045	2012-2011==0	0.0148	0.05633	0.263	1
				2014-2011==0	0.24799	0.05633	4.403	6.42E-05
				2015-2011==0	0.16247	0.05633	2.884	0.023532
				2014-2012==0	0.23318	0.05633	4.14	0.000209
				2015-2012==0	0.14766	0.05633	2.622	0.052521
				2015-2014==0	-0.08552	0.05633	-1.518	0.773691

Table S4.6. Results of the mixed effect models fitted to single-trait functional dispersion metrics after backward selection, and model choice based on the lowest AIC. LT precip – long-term precipitation; HY – hydrological year.

Traits/Func. groups	predictor	Std.coef	Std_error	df	t-test p-value	Rel. effect (%)	Marg. R^2
Life cycle	slope	0.1811	0.0851	6	0.0775	17.09	0.45
	Time	0.1681	0.0322	25	0.0000	15.87	
	LT_precip	0.0439	0.0851	6	0.6244	4.14	
	Time:LT_precip	-0.0786	0.0327	25	0.0240	7.41	
Growth-form	Tmax_HY	-0.0240	0.0063	23	0.0009	16.41	0.45
	LT_precip	-0.0111	0.0078	7	0.1968	7.62	
	Time	0.0020	0.0048	23	0.6859	1.35	
	Tmax_HY:LT_precip	-0.0145	0.0064	23	0.0335	9.93	
	LT_precip:Time	-0.0141	0.0045	23	0.0052	9.62	
Max. height	Tmin_HY	0.0845	0.0282	22	0.0066	9.15	0.52
	Tmax_HY	-0.0246	0.0381	22	0.5248	2.67	
	slope	0.0550	0.0484	6	0.2992	5.96	
	Time	0.0757	0.0273	22	0.0111	8.20	
	LT_precip	0.0150	0.0492	6	0.7711	1.62	
	Tmax_HY:slope	-0.1148	0.0348	22	0.0033	12.43	
	Tmax_HY:LT_precip	-0.1062	0.0360	22	0.0075	11.50	
SLA	Tmin_HY	0.0115	0.0045	25	0.0178	5.99	0.45
	Time	0.0153	0.0040	25	0.0008	7.97	
	LT_precip	0.0216	0.0099	5	0.0809	11.25	
	slope	0.0037	0.0069	5	0.6127	1.94	
	LT_precip:slope	0.0343	0.0134	5	0.0503	17.90	
Onset flower.	Tmax_HY	-0.0140	0.0197	24	0.4841	3.42	0.53
	LT_precip	0.0173	0.0198	6	0.4161	4.22	
	slope	-0.0554	0.0193	6	0.0286	13.50	
	Tmax_HY:LT_precip	0.0695	0.0224	24	0.0049	16.94	
	Tmax_HY:slope	0.0620	0.0225	24	0.0109	15.11	
Duration flower.	Tmax_HY	-0.0797	0.0248	25	0.0036	14.32	0.25
	Tmin_HY	-0.0589	0.0248	25	0.0256	10.59	
Dispersal strategy	Tmax_HY	-0.0687	0.0157	24	0.0002	18.87	0.53
	Time	0.0755	0.0147	24	0.0000	20.74	
	LT_precip	-0.0079	0.0148	7	0.6072	2.18	
	Time:LT_precip	-0.0399	0.0133	24	0.0062	10.96	
Seed mass	Precip_HY	0.0503	0.0080	23	0.0000	17.08	0.47
	Time	0.0431	0.0082	23	0.0000	14.65	
	Tmax_HY	-0.0170	0.0091	23	0.0742	5.77	
	LT_precip	0.0070	0.0133	7	0.6157	2.38	

Traits/Func. groups	predictor	Std.coef	Std_error	df	t-test p-value	Rel. effect (%)	Marg. R^2
	Tmax_HY:LT_precip	-0.0203	0.0082	23	0.0210	6.89	
Seed persistence	Tmin_HY	0.1201	0.0218	24	0.0000	26.43	0.58
	slope	0.0219	0.0269	7	0.4432	4.81	
	Time	0.0510	0.0192	24	0.0139	11.23	
	Tmin_HY:slope	0.0688	0.0217	24	0.0042	15.13	

Table S4.7. Relative effect of parameter estimates of model predictors and their interactions on the community-weighted means of the nine traits studied (except bulb growth-form and ecto and endozoochory dispersal strategies, for which models were not significant). Marginal and conditional R^2 of each model are presented. LT precip – long-term precipitation; SLA – specific leaf area.

		Short-term climate (ST)												
Trait	Func. Groups	Precip.	Tmax	Tmin	Slope	LT precip	Time	ST x Slope	ST x LT precip	Slope x Time	LT precip x Time	LT precip x Slope	Marg. R^2	Cond. R^2
Life cycle				8.84	13.46		19.06	10.93		9.98			0.62	0.70
Growth-form	Bulb													
	Erect		18.99										18.99	0.47
	Graminoid			19.54									19.54	0.38
	Prostrate		14.77										14.77	0.67
	Rosette	20.58		14.33		13.45							0.48	0.56
	Shrub			12.12	14.14		17.85	10.88		10.18			0.65	0.70
Max. height		18.38		21.93	3.67			7.35					0.51	0.60
SLA				14.44	21.02		15.10						0.51	0.61
Onset flowering					8.57	8.90						18.70	0.36	0.39
Duration flower.			17.80										0.18	0.18
Dispersal strategy	Anemochory	17.37		19.31	2.48			10.70					0.50	0.50
	Barochory	20.99		20.86	3.94			10.09					0.56	0.60
	Ectozoochory													
	Endozoochory													
Seed mass		1.45			5.33	4.94	0.48	4.23			4.26		0.21	0.82
Seed persistence		13.47		12.07	6.23			4.96					0.37	0.37
N-fixing ability														

Table S4.8. Relative effect of parameter estimates of model predictors and their interactions on the functional dispersion of the nine traits studied. Marginal and conditional R^2 of each model are presented. LT precip – long-term precipitation; SLA – specific leaf area.

	Short-term climate (ST)												
Trait	Precip.	Tmax	Tmin	Slope	LT precip	Time	ST x Slope	ST x LT precip	Slope x Time	LT precip x Time	LT precip x Slope	Marg. R^2	Cond. R^2
Life cycle				17.09	4.14	15.87				7.41		0.45	0.74
Life-form	25.32		18.99									0.44	0.68
Growth-form		16.41			7.62	1.35		9.93		9.62		0.45	0.66
Max. height		2.67	9.15	5.96	1.62	8.20	12.43	11.50				0.52	0.69
SLA			5.99	1.94	11.25	7.97					17.90	0.45	0.58
Onset flowering		3.42		13.50	4.22		15.11	16.94				0.53	0.53
Duration flowering		14.32	10.59									0.25	0.25
Dispersal strategy		18.87			2.18	20.74				10.96		0.53	0.55
Seed mass	17.08	5.77			2.38	14.65		6.89				0.47	0.75
Seed persistence			26.43	4.81		11.23	15.13					0.58	0.65
N-fixing ability													
Max. root depth				30.51								0.31	0.51

Chapter 5

Functional diversity responds more consistently to aridity than species diversity

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5 Functional diversity responds more consistently to aridity than species diversity

5.1 Abstract

Drylands are water-limited areas inhabited by 38% of the world's population. They are highly susceptible to desertification and land degradation. Climate change models predict an increase in aridity and in the extent of areas at risk of desertification. Previous studies reported a decline in primary production with increasing aridity in drylands; however, the response of species and functional trait diversity to aridity has not been fully explored. This response is particularly important since functional diversity closely reflects the influence of species traits on ecosystem functioning. Here we show how plant functional diversity changes with aridity in dryland ecosystems. We studied the plant community of 49 savanna-like dryland sites along a spatial aridity gradient, with a grassy-type understory representative of 52% of the vegetation physiognomies found in drylands. By computing plant functional diversity for eight relevant traits, we found a monotonic non-linear decrease in functional diversity with increasing aridity. This trend was unrelated to recent precipitation. Species diversity was not clearly related to aridity and its response was more dependent on recent precipitation. Our results support the use of functional, rather than species diversity as a reliable measurement of the impact of aridity on dryland ecosystems. According to our findings, an increase in aridity, particularly above a critical threshold, is likely to affect ecosystem functioning and thus the ability of dryland ecosystems to support more than one-third of the human population.

Keywords: climate change; drylands; ecosystem functioning; functional trait; grasslands; land degradation and desertification

5.2 Introduction

Drylands are characterized by an amount of rainfall below the evaporative demands imposed by high temperatures and solar radiation and are subject to high interannual climatic variability (MEA, 2005; Reynolds et al., 2007). They occupy 41% of the land surface, and include dry sub-humid, semi-arid, and arid areas classified according to the aridity index of United Nations Environment Programme (UNEP) (Middleton and Thomas, 1992) (range: 0.05–0.65). Water constraints limit the productivity of drylands, increasing their susceptibility to wind and water erosion and leading to low soil fertility. Land degradation and desertification already affect 10–20% of drylands worldwide and the area is likely to increase substantially as a result of population growth and climate change (MEA, 2005).

Despite their aridity, drylands support a rich biodiversity of plant and animal species (Davies et al., 2012) that have evolved to cope with the seasonal patterns of water availability. This biodiversity interacts with abiotic factors, enbling dryland ecosystems to maintain their multiple functions (e.g., productivity, nutrient fluxes) and their resilience (i.e., the ability to return to a previous state after disturbance), both of which are critical to ensure the provision of ecosystem services (MEA, 2005). As aridity increases, primary productivity decreases and nutrient cycling is affected (Delgado-Baquerizo et al., 2013b), causing direct and indirect impacts on ecosystem services delivery. According to climate change forecasts, these effects will worsen as aridity is projected to increase (MEA, 2005; Dai, 2013). However, the effects of increasing aridity on dryland ecosystems' biodiversity, remains unclear.

It has been suggested that a higher aridity may lead to lower species richness, because primary productivity falls and reduces the availability of resources for wildlife (Davies et al., 2012), although this assumption lacks scientific evidence. Species richness does not reflect species functional role on ecosystems, disregarding their functional uniqueness or redundancy within the community (Petchey and Gaston, 2006, Cadotte et al., 2011). Thus, information only about species richness does not provide insights about the effects of increasing aridity on ecosystem functioning (Cadotte et al., 2011). For instance, a recent work reported a positive effect of plant species richness on several ecosystem functions in water-limited ecosystems (Maestre et al., 2012a). However, this effect was largely modulated by changes in community composition and functional characteristics (Maestre et al., 2012b). Moreover, species richness may respond idiosyncratically or peak at intermediate levels of disturbance, potentially providing no signal of change (Mouillot et al., 2013). Species influence ecosystem processes *via* their functional traits (de Bello et al., 2010; Mouillot et al., 2011). Hence, a

functional approach in addition to a taxonomic one may be more revealing of the effect of aridity on plant communities and its consequences for ecosystem processes. It may enable the detection of critical abundance changes (e.g. of a set of traits under pressure) able to affect ecosystem functioning, being reactive even when species richness is not (Mouillot et al., 2013).

The diversity of functional traits is closely related to ecosystem functioning (Mouillot et al., 2011). Theory predicts that ecosystem functioning decreases with the decreasing diversity of species functional traits, due to a lower resource-use efficiency in heterogeneous environments (Mouillot et al., 2011). Functional diversity also allows the assessment of ecosystem resilience. The greater the presence of functionally similar species (higher redundancy), the higher the probability that disturbance-induced local extinctions of species will be compensated by the presence of similar species, ensuring higher ecosystem resilience (Pillar et al., 2013). Therefore, functional diversity may respond in a more predictable way to aridity than taxonomic diversity. However, the response of plant functional trait diversity specifically to aridity gradients has not been fully assessed (de Bello et al., 2006), particularly concerning the response of annual species (Gross et al., 2013). Annual species are an important component of dryland plant communities, particularly in grasslands, savannas, and open woodlands, often bearing most of the floristic diversity (Aronson et al., 1993, Noy-Meir, 1973). The herbaceous understory plays a decisive role in ecosystem functioning, namely in energy flow and nutrient cycling, because of the high turnover rate of its nutrient-rich and short-lived aboveground biomass (Gilliam, 2007).

In dryland plant communities dominated by annual species, variation in precipitation between years may affect plant productivity, species composition and diversity (Peco et al., 1998, Miranda et al., 2009), and plant functional traits (Carmona et al., 2012). In addition, carryover effects from the precipitation of the previous year(s) on plant diversity, either structural (e.g., through plant density) or biogeochemical (e.g., nutrient input by litter decay) (Sala et al., 2012), may also take place. Therefore, climatic fluctuations between years are likely to modulate aridity effect on plant communities. Soil characteristics can also ameliorate climate effects, since they largely determine water availability to plants, by determining infiltration, runoff, water-storage capacity, and evaporation (Noy-Meir, 1973).

In this work, we propose to evaluate the response of taxonomic and functional diversity of dryland plant communities to aridity, and assess how these responses are influenced by recent precipitation and soil characteristics. To assess this, we studied the plant community of Mediterranean Holm-oak woodlands, which are composed of a

sparse tree cover and an understory of semi-natural grasslands dominated by annual species, intermingled with shrubland patches. We studied eight plant traits related to plant establishment, nutrient uptake, reproduction, and regeneration, at the community-level, comprising annual and perennial species, along a spatial gradient of aridity with high-resolution. We specifically tested the following hypotheses: (i) functional diversity responds in a more predictable way to aridity than taxonomic diversity; (ii) taxonomic diversity is more affected by recent precipitation than functional diversity.

5.3 Material and methods

Field sampling

Field data were collected in 49 savanna-like dryland sites (southwestern Europe, Iberian Peninsula) dominated by Holm-oak (*Quercus ilex* L.) woodlands. Sampling sites were selected along an aridity gradient ranging from semi-arid to dry sub-humid according to the UNEP aridity index (AI) (Middleton and Thomas, 1992), where AI is defined as the ratio of mean annual precipitation to annual potential evapotranspiration. AI values for 1950–2000 were retrieved from a global database (Trabucco and Zomer, 2009). We surveyed the understory of Holm-oak woodlands consisting of semi-natural grasslands of mainly annual species with shrubland patches. Before the selection of the sampling sites based on aridity, a pre-selection of 'suitable sites' was made to ensure homogenization and avoid confounding effects from other factors following several criteria: land-use intensity (moderate to low extensive grazing intensity and no agricultural activity in recent years), altitude (150-300 m), soil dominantly acidic (pH<6.5) and dominated by sedimentary and metamorphic lithology, and absence of fire in the last decades. Low grazing intensity and the absence of recent agricultural activities was further confirmed in the field by empirically inspecting the appearance of the herbaceous vegetation, the absence of recent soil tillage, and the general amount of ungulate pellets. Within each site, plant sampling was conducted avoiding drainage lines and flooding surfaces. Species cover was estimated by the point–intercept method (Nunes et al., 2014), as the proportion of points intercepted along six 20-m transects with points spaced every 50 cm. At each point a 5mm diameter rod was put in the soil making a 90◦ angle and all plant species which contacted with the rod were recorded. The same species was recorded only once at each point. Measurements were conducted between April and the beginning of June of 2012 when vegetation reaches its biomass peak. The year 2012 was drier than the 50-year mean annual precipitation (370±61 mm vs. 561±27 mm).

Given the amount of sites sampled, whenever possible, sampling was conducted from south (where spring growth usually begins earlier) to north, to avoid any phenological lag among sites. At each site, a composite soil sample (0–10 cm depth), consisting of five sub-samples randomly collected and homogenized, was analyzed to measure soil organic-matter content (%) by the gravimetric method. According to the information derived from the Portuguese Environment Agency (APA) (http://www.apambiente.pt) (Atlas Digital do Ambiente, 2011), two main soil types were found along the study area: leptosols (azonal, very shallow and stony soils with high internal drainage), and luvisols (soils with a higher clay content in the subsoil than in the topsoil) (Schad and Spaargaren, 2006).

Diversity metrics

To assess plant diversity at each site we measured: i) species richness (Richness), ii) taxonomic diversity (TD), computed as the Simpson diversity index, and iii) functional diversity (FD). Dominant species (attaining $\geqslant$80% of the relative cover) were used to compute functional diversity, following recommended protocols (Pakeman and Quested, 2007). The 95 most abundant species were used from the 256 species recorded. Traits were measured directly in the field following standard protocols (Cornelissen et al., 2003) or derived from other sources (Supplementary Table S5.1). From over 30 plant traits considered initially, we selected the 11 for which information for most of the species was available. Finally, only the eight functional traits responsive to aridity and reflecting plant strategies related to establishment, persistence, regeneration, and dispersal were used: life cycle, height, specific leaf area, seed persistence, dispersal mode, flowering onset, flowering duration, and nitrogen-fixing ability (Supplementary Table S1). Ordinal traits (life cycle and seed persistence) were first transformed into numerical values; categorical traits (dispersal mode and nitrogen-fixing ability) were coded as binary variables (Supplementary Table S5.1). To describe functional diversity we calculated the functional dispersion (Laliberté and Legendre, 2010), which expresses the extent of trait differences within a community (or dispersion around the mean trait value), and is closely related to Rao's quadratic entropy. Functional dispersion is the weighted mean distance, in multidimensional trait space, of individual species from the weighted centroid of all species, where weights correspond to relative species abundances. We computed functional dispersion both for single traits (FDsingle, with single-trait metrics later combined in one multivariate matrix) and for all traits combined (FDall). The Gower distance, which can handle continuous, ordinal, and categorical

variables as well as missing values, was used. All calculations were done with the dbFD function of the FD package (Laliberté et al., 2014) in R (R Core Team, 2015).

Choice of modeling variables

We first analyzed the relation of plant diversity metrics (taxonomic and functional) with aridity (Table 5.1), since aridity reflects long-term water limitation, the primary determinant of ecosystem functioning in drylands, and is the desertification indicator used by the United Nations Convention to Combat Desertification (Middleton and Thomas, 1992). At our study sites, the aridity index (AI) was closely related to mean annual precipitation (Spearman's r=0.95, P<0.001) and mean annual temperature (Spearman's r=-0.75, P<0.001). Precipitations of the current and previous hydrological-years (cumulative precipitation from October to September) were used as short-term climatic predictors (Table 5.1). They were derived from the Instituto Português do Mar e da Atmosfera data (https://www.ipma.pt/). Climate stations located up to 72 km from the sampling sites were selected for the analysis. For each month, the semi-variogram of total precipitation values was calculated and a model was manually fitted to it. Subsequently, the monthly precipitation values were interpolated using ordinary kriging methodology (Costa et al., 2008). Variograms were calculated using geoMS, Geostatistical Modelling Software (CERENA, 2000) and the maps were interpolated using ArcGIS 10.1 (ESRI, 2010) within the study region. Finally, using the interpolated maps, the total annual values of precipitation were retrieved for each sampling site. Soil type (Schad and Spaargaren, 2006, Atlas Digital do Ambiente, 2011) and organic matter content were also used as predictors (Table 5.1).

Table 5.1. Description and statistics of predictor variables used in model selection.

Variable	Description	Unit	Mean ± SD	Min	Max
Aridity	Aridity index (UNESCO, 1979)	–	0.48±0.04	0.42	0.56
Current HY	Current hydrological year (cumulative precipitation from October to September)	mm	174.9±66.2	79.2	290.8
Previous HY	Previous hydrological year (cumulative precipitation from October to September)	mm	400.5±113.7	212.4	667.4
Soil OM	Soil organic matter	%	6.8±2.9	2.3	15.6
Soil type	Leptosols or luvisols (IUSS Working Group WRB, 2006)				

Statistical analyses

Site-averaged species cover, and mean trait values per species, were used in the statistical analyses. Both univariate (Richness, TD, FDall), and multivariate (FDsingle for each of the eight traits combined in one matrix) responses were analyzed. Bray-Curtis dissimilarities for plant trait data were used in a permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations to select significant variables, starting with the full model with five predictor variables (after checking for their colinearity). For a univariate response, this approach resembles a multiple regression. To test for monotonic but not necessarily linear relationships, the final models were confirmed with Mantel tests (9999 permutations) based on Spearman correlations using Euclidean distances for predictor variables (previously standardized). The effect of each predictor variable, alone or in combination, was analyzed using partial Mantel tests on the final models, testing the predictor variables individually, with the remaining variables serving as covariates (Supplementary Table S5.2), and by applying variance partitioning techniques. All analyses were performed using the 'vegan' package (Oksanen et al., 2016) in R (R Core Team, 2015).

5.4 Results and Discussion

The results showed a monotonic non-linear decrease in multi-trait functional diversity with increasing aridity (Fig. 5.1c). The decrease in functional diversity was more pronounced after being crossed a critical aridity level (AI < 0.46–0.50) (Fig. 5.1c). A non-linear fit was confirmed with polynomial regression with a cubic term for aridity, which performed better than the linear model ($P<0.05$; $F=4.20$). From the predictor variables considered, only aridity had significant explanatory power for univariate multi-trait functional diversity ($P<0.01$; $R^2=0.16$). It was also the most important variable explaining multivariate single-trait functional diversity ($P<0.001$; $R^2=0.14$; Table 5.2). This monotonic decreasing trend with aridity was further confirmed with rank Mantel tests (Table S5.2) and was also examined and confirmed for each trait analyzed individually. The decrease in functional diversity with increasing aridity implies lower dissimilarity of plant functional traits with more arid conditions. In drier sites, more stressful conditions cause a selection of a narrower variety of plant strategies, probably because of habitat filtering; as such, species share traits more adapted to aridity. Thus, among the plant traits studied, aridity might select, for instance, for limited longevity (annual life cycle) (Noy-Meir, 1973), a shorter period of vegetative growth, and earlier flowering (Petru et

al., 2006). Greater aridity may also favor the persistence of seeds capable of withstanding longer drought events (Peco et al., 2003), during which species with short-lived seed banks tend to disappear. In less arid sites, plants with these traits may coexist with those of medium to high longevity, later flowering, and low seed persistence, with higher trait diversity linked to higher niche differentiation.

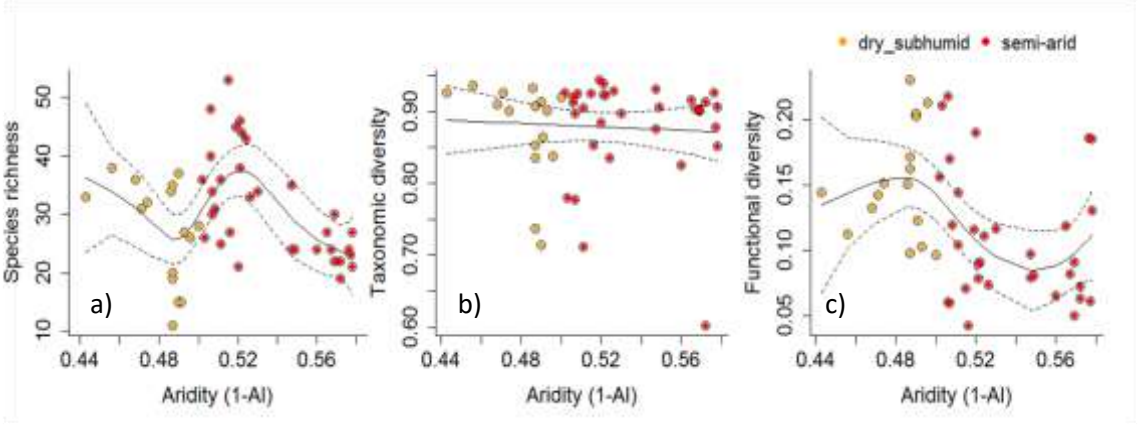

Figure 5.1. Response of species diversity and functional diversity to aridity. (a) Species richness; (b) taxonomic diversity (computed as Simpson diversity index); (c) functional diversity (computed as functional dispersion (Laliberté and Legendre, 2010) for eight traits combined - FDall). Aridity is based on the aridity index (AI) of the United Nations Environment Programme (UNEP)(Middleton and Thomas, 1992). To improve clarity, we present 1-AI, so that higher values represent drier conditions. Solid lines represent non-linear spline functions; dashed lines indicate 95% confidence intervals, estimated using generalized additive models.

Functional diversity responded to long-term climate conditions. In fact, in this study, only the relation between plant functional diversity and aridity, a long-term climatic variable, was significant, suggesting that factors underlying functional diversity operate over the long-term. Examples of these factors are: i) soil features, including water storage capacity during dry periods, and ii) plant seed banks. Soil features may reflect the climatic history, e.g., the effect of aridity on soil nutrients (Delgado-Baquerizo et al., 2013a; Delgado-Baquerizo et al., 2013b), and biotic legacies, e.g., the effect of previous plant cover and management strategies on nutrient inputs into the soil (Sala et al., 2012). Plant seed banks depend on: i) whether the climatic conditions of the previous year(s) allowed a certain species to germinate, flower, and produce new seeds and ii) the persistence of those seeds in the soil, which can last for several years (Peco et al., 2003). These long-term factors condition the effects of recent precipitation (favorable or not) on vegetation.

Table 5.2. Final models obtained after PERMANOVA to analyze the effects of the predictor variables on species richness (Richness), taxonomic diversity (TD), multivariate single-trait functional diversity (FDsingle), and univariate multi-trait functional diversity (FDall). The final model for each response variable was obtained after deletion of the predictor variables one at a time, followed by a comparison of the depleted model with the previous and the full models. The predictor variables are described in Table 5.1.

	Richness			TD	FD_{single}			FD_{all}		
	F	*P*	R^2		*F*	*P*	R^2	*F*	*P*	R^2
Aridity	ns	–	–	ns	8.05	<0.001	0.14	9.09	0.003	0.16
Curr. HY	18.1	<0.001	0.26	ns	ns	–	–	ns	–	–
Prev. HY	ns	–	–	ns	3.35	0.039	0.06	ns	–	–
Soil OM	5.00	0.023	0.07	ns	ns	–	–	ns	–	–
Soil type	ns	–	–	ns	ns	–	–	ns	–	–

Species richness responded to recent precipitation and was not markedly related to aridity (Fig. 5.1a, Table 5.2). It was better explained by variables acting over the shorter-term, such as precipitation in the current hydrological year ($P<0.001$; $R^2=0.26$), and also by soil organic-matter content ($P<0.05$; $R^2=0.07$). None of the predictor variables was related to taxonomic diversity (Fig. 5.1b, Table 5.2). Species richness seemed to reflect the effects of recent precipitation patterns, as previously reported for annual plant communities (Peco et al., 1998), which dominated our study area. There was also no effect of previous-year precipitation, which in this study was in the "normal" range, on species richness. Hence, previous-year precipitation did not place substantial constraints on the plant community in the following year, when sampling took place. Plant functional diversity did not correlate with taxonomic diversity (Spearman's $r=-0.14$, $P>0.1$) or species richness (Spearman's $r=-0.16$, $P>0.1$). The decoupled response of functional and species diversity to aridity points to a high functional redundancy in dryland vegetation. Although there may have been changes in plant species between years, functional diversity was maintained, suggesting the presence of different species with the same function and thus a high functional redundancy in these ecosystems. The consistency of functional diversity during climatically different years in Mediterranean grasslands was previously suggested (Carmona et al., 2012). It points to the maintenance of ecosystem processes between years and thus to a high resilience of dryland ecosystems to interannual climatic changes.

Lower functional diversity with increasing aridity probably implies a reduction in ecosystem functioning. Given the reduction in trait dissimilarity at drier sites (Fig. 5.1c), a reduction in ecosystem processes can be expected, e.g., in primary productivity or

decomposition rates, as reported by other authors (Mouillot et al., 2011). These in turn may manifest as a lower capacity of the ecosystem to provide services such as carbon sequestration, soil fertility, or climate and water regulation (de Bello et al., 2010), entailing socio-economic consequences. Dryland ecosystem functioning appear to rely not on species richness alone, which may vary considerably between years, but on their functional diversity, in accordance with results from manipulative experiments (Mouillot et al., 2011). Our results are particularly important in view of the relevance of natural and semi-natural grasslands dominated by annual species and their associated savannas and shrublands, which together comprise 52% of dryland areas worldwide (Reynolds et al., 2007).

Functional diversity is a more consistent indicator of the impact of aridity than species diversity. It showed a monotonic change along the aridity gradient (consistently decreasing or remaining unchanged with increasing aridity), thus integrating plant community responses in a predictable manner. Given the stability of plant functional diversity despite interannual fluctuations in other biodiversity components (e.g., species richness, it is a more informative measure of the functional state of dryland ecosystems. Species loss, traditionally used for ecosystem monitoring, may indeed: i) reflect short to medium-term reversible trends rather than integrated ecosystem-level responses and ii) respond idiosyncratically or unimodally, thus failing to reveal disturbances (Mouillot et al., 2013). Accordingly, by detecting critical abundance changes (e.g., in a set of traits under pressure) that may affect ecosystem functioning, functional rather than species diversity is an appropriate indicator of the impacts of aridity.

We provide the first study showing that plant functional diversity is a more reliable indicator of the impact of aridity on dryland ecosystems than species diversity. Moreover, the use a high-resolution gradient within the aridity range it encompassed, allowed us to understand the "shape" of the plant functional response. Our results showed that in dryland ecosystems, plant functional diversity is shaped by long-term factors, despite interannual variations in other diversity components (e.g., species richness) linked to short-term climatic fluctuations. Consequently, there is likely to be a lag in the response of functional diversity to the altered precipitation patterns and temperature increases predicted by climate change models. Nevertheless, according to our findings, an increase in aridity (Dai, 2013) should reduce plant functional diversity, especially after a critical aridity threshold is crossed (AI<0.46–0.50). This would reduce ecosystem functioning and thus the ability of dryland ecosystems to support more than one-third of the human population.

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Supporting Information

Table S5.1. Description of the functional traits considered in the study. Sources used for trait data (1-20).

	Trait	Type	Categories/units	Function
Ecological strategy	Life cycle	ordinal	Annual, biennial or perennial	Soil protection, biomass production, nutrient cycling, resistance to disturbance
	Max. height	cont.	cm	Dispersal distance, light capture, above-ground competition, resistance to disturbance
	SLA	cont.	mm^2/mg	Photosynthesis and growth, leaf longevity, decomposition
Nutrient uptake	N-fixing ability	binary	Yes or no	Resource acquisition, nutrient cycling
Reproductive	Onset of flowering		Initial month	Phenological and reproductive strategy, stress avoidance
	Duration of flowering		Number of months	
Regenerative	Dispersal strategy	binary	anemochory or other	Dispersal ability under spatial and temporal heterogeneity, stability (species pool)
	Seed persistence	ordinal	Transient (<1 year), short-term persistence (1<years<5), long-term persistence (> 5 years)	Diversity 'storage', dispersal ability under unpredictable/harsh conditions

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Table S5.2. Correlation coefficients (r) between plant trait data and predictor variables as measured with simple and partial Mantel tests for species richness, taxonomic diversity (TD), multivariate single-trait functional diversity (FDsingle), and multi-trait functional diversity (FDall). Mantel tests based on Spearman correlations between the distance matrices were used to test for monotonic relationships (9999 permutations). The predictor variables are described in Table 5.1.

	Richness		TD	FD_{single}		FD_{all}	
	r	*P*		*r*	*P*	*r*	*P*
Aridity	–	–	–	0.17	0.002	11.0	0.014
with previous HY partialled out	–	–	–	0.18	0.004	–	–
Current HY	0.21	<0.001	–	–	–	–	–
with soil OM partialled out	0.21	<0.001	–	–	–	–	–
Previous HY	–	–	–	0.05	ns	–	–
with aridity partialled out	–	–	–	0.03	ns	–	–
Soil OM	0.10	ns	–	–	–	–	–
with current HY partialled out	0.11	0.047	–	–	–	–	–

HY, hydrological year; OM, organic matter

Chapter 6

The importance of topo-edaphic drivers on shrub encroachment and associated functional changes in Mediterranean drylands

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6 The importance of topo-edaphic drivers on shrub encroachment and associated functional changes in Mediterranean drylands

6.1 Abstract

Shrub encroachment is a worldwide phenomenon affecting mainly drylands. Previous works already identified a combination of multiple factors responsible for shrub encroachment. However, the relative effects of climate and of topographic and edaphic factors on shrub encroachment, and on associated plant functional changes, are clearly lacking evidence from Mediterranean Basin drylands. We propose to overcome this knowledge gap by investigating how these factors interact to affect shrub encroachment. To do so, we studied savanna-like Holm-oak woodlands along a regional climatic gradient. We specifically aimed at assessing (i) how climatic, topographic and edaphic factors influence relative shrub cover (RSC) and (ii) their direct and indirect effects (via RSC) on community mean and range of 12 functional traits related to plant establishment, persistence, regeneration, and dispersal. We found that under low-intensity and similar land management, local topo-edaphic factors, namely slope variations and soil C:N ratio, were the most important drivers of shrub encroachment, determining the main functional changes in the studied communities. Climate acted as an additional environmental filter, modulating the effect of local topo-edaphic factors; jointly these factors accounted for 52% of the variation in relative shrub cover. Still, aridity, summer precipitation and winter temperature had a direct effect on particular plant traits related to flowering and dispersal strategies. Our findings imply that management actions aiming at reversing shrub encroachment in Mediterranean grasslands need to consider topo-edaphic factors (e.g. involve alterations in terrain structure to change water and nutrient flow pathways and accumulation) to be more cost-effective and sustainable. In addition, our results suggest that altered precipitation regimes and an overall increase in aridity forecasted for the Mediterranean Basin may not have a major impact *per se* on shrub encroachment, but may directly affect other traits (e.g. shorten flowering duration) and reduce functional diversity for some traits, thus compromising ecosystem functioning.

Keywords: aridity; functional traits; Holm-oak woodlands; slope; soil C:N; structural equation models

6.2 Introduction

Shrub encroachment, defined as the increase in density, cover and biomass of native woody species (Van Auken, 2009), has been topic of numerous publications in the last decades e.g. (Roques et al., 2001, Maestre et al., 2009, Van Auken, 2009) and subject to an intensive debate in recent literature (Eldridge et al., 2011, Sala and Maestre, 2014, Eldridge and Soliveres, 2015, Maestre et al., 2016). This is a world-wide phenomenon, reported mostly for dryland ecosystems, e.g. in North America (Van Auken, 2009), Australia (Eldridge and Soliveres, 2015), South Africa (Roques et al., 2001) and in the Mediterranean Basin (Castro and Freitas, 2009, Maestre et al., 2009, Caldeira et al., 2015). It is the result of both natural and human-induced processes through a combination of multiple factors that are difficult to disentangle, leading to apparently contradictory results. Long-term overgrazing has been frequently pointed out as being (directly or indirectly) responsible for a hard-to-reverse increase in shrub cover in grasslands in western United States or in southern Africa drylands (Schlesinger et al., 1990, Roques et al., 2001, MEA, 2005, Van Auken, 2009). Contrastingly, in other parts of the world, e.g. in the Mediterranean Basin, it is suggested that shrub encroachment is largely a consequence of grazing exclusion (Castro and Freitas, 2009). This phenomenon has been also associated to past shrub clearing, reduced fire frequency, increases in CO^2, N deposition and long-term climate change (see (Eldridge et al., 2011) for a review).

Climate has a major influence on the structure of plant communities, not only over time, but also along space. At a larger geographic scale, increasing precipitation largely explains transitions between grassland, savanna and woodland (Hirota et al., 2011). At a local scale, variations in woody cover not strictly induced by land management (e.g. shrub clearing), are influenced by the interaction between climate and local topo-edaphic factors (Colgan et al., 2012, Sala and Maestre, 2014), particularly in water-limited ecosystems (Gómez-Plaza et al., 2001). Finally, biotic-abiotic interactions may also influence shrub cover through positive feed-backs. It has been suggested that shrub encroachment may be a self-reinforcing process: shrub colonization would lead to a more heterogeneous distribution of soil water and nutrients, thus promoting further shrub encroachment (Schlesinger et al., 1990). In short, shrub encroachment seems to depend on multiple factors, varying with the historical management (e.g. grazing pressure), environmental conditions (e.g. climate, soil properties, topography) and the scale of analysis (e.g. landscape or patch level) (Eldridge et al., 2011, Eldridge and Soliveres, 2015).

Another source of controversy involves the consequences of shrub encroachment for ecosystem functioning. Some authors see it as a land degradation process (Schlesinger et al., 1990, MEA, 2005), frequently from the point of view of livestock production which is a primary use of drylands. Other authors argue it may improve some aspects of ecosystem functioning, like carbon sequestration (Daryanto et al., 2013), vascular plant richness, soil fertility and N mineralization rate (Maestre et al., 2009, Gómez-Rey et al., 2013) (see (Eldridge et al., 2011) for a synthesis). A recent work found higher plant diversity, soil fertility and nutrient pools at intermediate levels of relative woody cover (41–60%) in drylands, particularly towards wetter environments (Soliveres et al., 2014). One of the conclusions that emerged from these apparent contradictions was that the effect of shrub encroachment for ecosystem functioning largely depends on the functional traits of the woody and herbaceous species involved (Soliveres et al., 2014, Maestre et al., 2016). This is not surprising at all, considering that functional traits are the means by which species influence ecosystem processes and associated ecosystem services (Diaz et al., 2007, de Bello et al., 2010).

Functional traits are most often described by their mean and range assessed at the community level (Diaz et al., 2007). The community mean reflects the traits of the dominant species (Garnier et al., 2007), which are expected to largely determine ecosystem processes (Grime, 1998). Trait range reflects the degree of functional dissimilarity within the community (Laliberte and Legendre, 2010), and a higher trait range is expected to lead to increased complementarity in resource use between species and thus to increased ecosystem functioning (Tilman et al., 1997). The classification of plants as being shrubs or herbaceous species is based on a series of traits, e.g. plant size, the presence of a woody stem, life-span, root depth and specific leaf area, which co-vary among the two growth-forms (Díaz et al., 2016). Similarly, functional changes in the plant community due to shrub encroachment should involve a series of co-varying traits, reflecting trade-offs in resource allocation patterns (Díaz et al., 2016).

Previous studies addressed the effects of multiple environmental factors on shrub encroachment, mainly from outside of the Mediterranean Basin (Roques et al., 2001, D'odorico et al., 2012). However, to our knowledge, none has quantified jointly the relative effects of climate and topo-edaphic factors on shrub encroachment and associated changes in community functional traits in Mediterranean Basin drylands. We propose to overcome this knowledge gap by studying the plant communities of savanna-like Holm-oak woodlands along a spatial climatic gradient, comprising sites with varying topo-edaphic characteristics. Hence, we adopted a ‘space-for-time substitution’

approach, assessing changes along space to infer changes over time (Blois et al., 2013). Holm-oak woodlands consist of a sparse tree cover with an understory of semi-natural grasslands intermingled with shrubland patches, and have a traditional low intensity silvo-pastoral use. Specifically, we assessed (i) the relative effects of climatic, topographic and edaphic factors on the increase of relative shrub cover; (ii) the direct and indirect effects (via relative shrub cover) of these factors on the mean and range of plant community functional traits. We studied 12 traits reflecting plant strategies related to establishment, persistence, regeneration, and dispersal. To test direct and indirect effects of environmental drivers on the plant community we built an a priori causal model based on our results and on observations of previous studies, using structural equation modelling (SEM model) (Grace et al., 2012). Under low-intensity and similar land management, climate and topo-edaphic variables are expected to exert a strong control on relative shrub cover (Sala and Maestre, 2014) at a regional and local scale, respectively. Our first hypothesis is that local topo-edaphic factors are as important as climate, as drivers of shrub encroachment. Grass and shrub-dominated communities are expected to differ considerably in functional traits means and range. Because functional traits are linked to ecosystem processes (e.g. primary productivity, nutrient decomposition rates) (de Bello et al., 2010), this information can provide clues about the consequences of shrub encroachment for ecosystem functioning. In addition, it is important to disentangle which functional consequences are mostly driven by local environmental factors, from those due to regional filters, i.e. climatic variables. Our second hypothesis is that the community response to local environmental filters via changes in relative shrub cover (i.e. indirectly) will affect a different set of functional traits (or axis of functional specialization) than that due to regional climatic filters.

6.3 Methods

Study sites and environmental variables

This study was carried out in southwestern Iberian Peninsula, Portugal (Alentejo). Field data were collected in 54 Mediterranean dryland sites dominated by savanna-like Holm-oak woodlands (*Quercus ilex* L. subsp. *rotundifolia*) along a regional climatic gradient, comprising semi-arid and dry sub-humid climates (Fig. S6.1). Sites were selected from the plots surveyed for the National Forest Inventory 2005/06 (AFN, 2010). We selected plots occupied by Holm-oak woodlands, at an altitude between 150 and 300

m, with dominantly acidic soil (pH<6.5) laying on sedimentary and metamorphic rocks, and without fire records, to avoid confounding effects of these factors (AFN, 2010; Atlas Digital do Ambiente, 2011). Afterwards, a stratified random selection of sites along a macroclimatic gradient was made based on the aridity index (Fig. S6.2). We used the aridity index (AI) of the United Nations (Middleton and Thomas, 1992) representing the ratio of mean annual precipitation to annual potential evapotranspiration. AI data for the period 1950–2000 were retrieved from the global aridity database (http://www.cgiar-csi.org/data/global-aridity-and-pet-database) (Trabucco and Zomer, 2009), and ranged from 0.42 to 0.56 along the study area (Fig. S6.1). Of these possible sites, we further selected the ones with moderate to low grazing intensity and no agricultural activities over the last five years. The latter conditions were empirically verified in the field by inspecting evidence of grazing in vegetation, the amount of ungulate pellets, and absence of recent soil tillage. The total number of selected sites was 54. The sampled sites were Holm-oak woodlands with a sparse tree cover and variable proportions of semi-natural grasslands intermingled with shrubland patches (shrub cover ranging from 0% to 87%, average 16%).

Each sampling site was characterized by a set of 19 climatic variables comprising temperature and precipitation metrics extracted from Worldclim database with a 1 km2 resolution (Hijmans et al., 2005) (Table S6.1). In addition, to summarize the topographic characteristics of each sampling site, three topographic variables were computed based on digital elevation models with 10 m resolution. We used local slope values, the standard deviation of the slope within a 250 m buffer around the sampling site centroid, and a topographic wetness index (Sørensen et al., 2005), to quantify topographic control on hydrological processes (Table S6.1). Soil characteristics at each site were measured on soil samples collected from the upper 10 cm (composite sample of 5 subsamples) using standard procedures. Soil texture (% of sand, clay and silt) and nutrient content (soil organic matter, soil N, and C:N ratio) were determined (Table S6.1). After exploring the correlation among the climatic, topographic and edaphic descriptors at each site, and because many of them were strongly correlated (Tables S6.2 and S6.3), we latter summarized them into 8 variables showing a Pearson´s r correlation <0.70 between them to be used in subsequent analysis (Table 6.1).

Vegetation sampling and community trait metrics

Plant sampling was conducted at the peak of standing biomass (April-June 2012) using the point–intercept method, avoiding drainage lines and flooding surfaces. At each

site, species cover was estimated along six 20 m transects systematically arranged over an area of ca. 1000 m2 and 10 m apart from each other, with points spaced every 50 cm (246 points per site) (for further detail on the sampling scheme see (Nunes et al., 2014). At each point, a rod of 5 mm diameter was placed in the ground with a 90° angle. All plant species touching the rod were recorded and the same species was recorded only once at each point. Species cover was calculated as the proportion of points intercepted per transect. Sampling took place in a relatively dry year (370±61 mm) when compared to the 50-year mean annual precipitation of the sampling sites altogether (561±27 mm). To characterize functional traits within the plant communities, we used the dominant species attaining ⩾80% of the relative cover in decreasing order of relative cover within single sites (Pakeman and Quested, 2007) (95 species overall, and 30±9 per site). We selected 12 plant traits reflecting plant strategies related to establishment, persistence, regeneration, and dispersal (Table S6.4). Trait information was obtained either through direct measurements in the field following standard protocols (Pérez-Harguindeguy et al., 2013), or derived from various bibliographic sources, or both (Table S6.4). We calculated trait mean and range through the community-weighted-mean (CWM) (Garnier et al., 2007) and functional dispersion (FDis) (Laliberté and Legendre, 2010), respectively. The CWM corresponds to the average trait value in a community weighted by the relative abundance of the species carrying each value (Garnier et al., 2007). FDis is calculated as the weighted mean distance, in multidimensional trait space, of individual species from the weighted centroid of all species, where weights correspond to species relative abundances (Laliberté and Legendre, 2010). Mean trait values per species were used to compute functional diversity metrics, since we were focused on the turnover between sites and not on intraspecific trait variability. Continuous traits were log transformed prior to analysis. The Gower distance was used in calculations because it can handle continuous, ordinal, and categorical variables, as well as missing values. All calculations were done with the dbFD function of the FD package (Laliberté et al., 2014) in R (R core Team, 2015).

Statistical analyses

We first analysed relative shrub cover through linear regression using the eight selected environmental variables as predictors (aridity index, summer precipitation, winter temperature, slope standard deviation, topographic wetness index, soil sand content, soil organic matter and C:N ratio), as well as two-way interactions among them (Table 6.1). We also included a quadratic term for aridity, as the response of the plant

community to aridity is not necessarily linear (Gross et al., 2013). Correlation among the predictors used had been previously checked to avoid multicollinearity, and was below Pearson´s r 0.7 in all cases (Tables S6.2 and S6.3). We used a multi-model inference approach and the best model was selected among several alternative models based on the Akaike information criterion (AIC). Afterwards, we built regression models for the functional trait metrics using the same predictors and procedure. We first explored the correlation among functional trait metrics calculated for the different traits and, because some were strongly correlated (Tables S6.5 and S6.6), we conducted a principal component analyses (PCA) using the CWM and FDis values of all the traits measured. These analyses were done separately for CWM and FDis, based on correlation matrices. This procedure allowed us to summarize trait variation, and identify the most informative axes of functional specialization (or plant strategy spectrum) along which traits co-vary (Díaz et al., 2016). We then used the two first PCA components of each analysis as a measure of the CWM and the FDis of each community, i.e., as response variables (see (Valencia et al., 2015) for a similar approach). Then, we used a variance decomposition analysis based on the best models selected to evaluate the relative importance of climatic and topo-edaphic predictors and their interactions, as drivers of the variation found in each response variable (Dubuis et al., 2013). We thereby obtained the percentage of variance explained by each group of predictors.

Finally, to identify the mechanisms that control shrub encroachment, and to test direct and indirect effects (i.e., via changes in relative shrub cover) of climatic and topo-edaphic factors on the functional traits of the plant community, we used structural equation modelling (SEM). This approach is well suited for studying hypotheses about multiple processes operating in systems with complex causal connections (Grace et al., 2012). It allows to partition causal influences among multiple variables, and thus to separate the direct and indirect effects of the predictors included in a model and estimate the strengths of these multiple effects. We established an a priori model based on our current knowledge and on the best regression models previously built for each response variable. We hypothesized that (i) climatic and topo-edaphic variables would directly affect relative shrub cover and the functional characteristics of the plant community and (ii) changes in functional metrics would be largely mediated by changes in relative shrub cover. Although we acknowledge that other a priori model structures could be possible, and that inferring cause-effect relationships from observational studies has its pitfalls, the metrics of goodness-of-fit of our model, and ecological evidences drawn from ours and other studies, clearly support the plausibility of the relations among variables we

propose. Before modeling, we examined the distributions of all our endogenous variables (i.e. response variables), and tested their normality. The first component of the PCA on CWM values was normalized (z-score) before analyses. To test the overall fit of SEM models we used the chi-square statistic and its significance (note that the model is rejected if p-value<0.05), the comparative fit index (CFI>0.9), the root mean square error of approximation index (RMSEA<0.10) and the standardized root mean square residual (SRMR<0.10). Standardized path coefficients estimated by maximum likelihood were used to measure the direct and indirect effects of the predictors (Grace et al., 2012). These coefficients are interpreted as the size of an effect that one variable exerts upon another. SEM models were fit using the Lavaan package (Rosseel, 2012). All the analysis were performed under R statistical environment (R Core Team, 2015). All the predictors used in modeling were standardized and normalized (z-score) before analyses.

Table 6.1. Environmental variables selected, their units and range in the study area.

Environmental variables	Description	Range and units
Climatic		
Aridity index	Ratio of mean annual precipitation to annual potential evapotranspiration, for the period 1950–2000	0.42 – 0.56 (unitless)
Summer precipitation	Precipitation of driest quarter	17 – 34 mm
Winter temperature	Mean temperature of the coldest quarter	9.4 – 11.3 ºC
Topographic		
Slope standard deviation (slope SD)	Standard deviation of the slope within a 250 m buffer around the sampling site (based on digital elevation models, 10m resolution)	1.16 – 14.69 º
Topographic wetness index (TWI)	Ln [(upslope area)/local slope], used to quantify topographic control on hydrological processes	6.32 – 14.44 (unitless)
Edaphic		
SOM	Soil organic matter content	2.3 – 15.6 %
C:N ratio	Soil Carbon/Nitrogen ratio	6.8 – 18.1 (unitless)
Sand	Soil sand content	17.9 – 71.4 %

6.4 Results

Variation in relative shrub cover (RSC) was mostly explained by topo-edaphic variables (64% of the adj. R^2=0.52), increasing with slope standard deviation and soil C:N ratio, which showed additive effects (Table 6.2, Figure 6.1). Lower aridity (higher aridity index) and summer precipitation also contributed to higher RSC, although the climatic predictors overall explained a small proportion of its variation along the climatic gradient either per se (15%), or in interaction with topo-edaphic factors (21%) (Table 6.2).

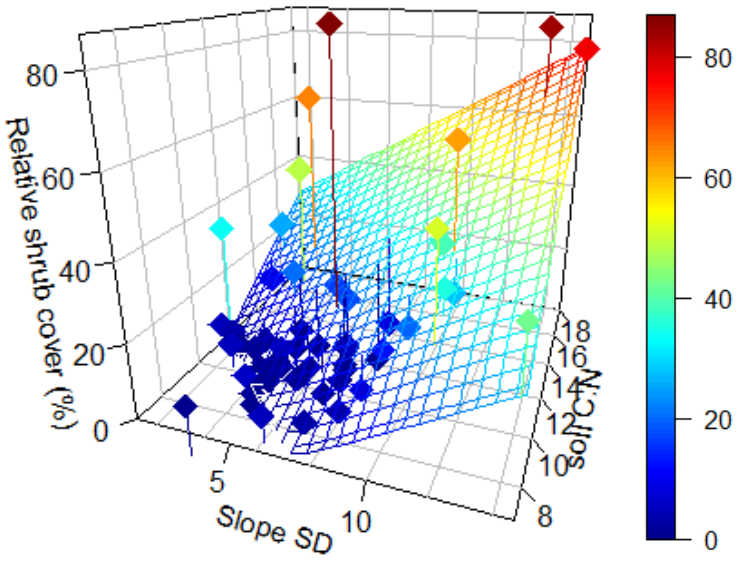

Figure 6.1. Relative shrub cover variation with slope standard deviation (slope SD) and soil C:N ratio. Planes represent the predicted values of a linear regression fitted to both variables. The colors of the predicted planes change from blue (low values of relative shrub cover) to red (high values of relative shrub cover).

Table 6.2. Resume of the best models selected for each response variable (see Tables S7 and S8 for further detail). The sign of the coefficients of the selected predictors in each model is indicated. The proportion of variance explained by topo-edaphic and climatic predictors (and by both) was calculated using a variance decomposition analysis based on each model.

	Predictors										Proportion of the explained variance (%)		
	Topo-edaphic					Climatic			Topo-edaphic x Climatic				
Response variables	Slope SD	Soil C/N	Slope SD x Soil C/N	Sand	soil C/N x Sand	Aridity	Summer precip.	Winter temp.	Slope SD x Summer precip.	Ad.R^2	Topo-edaphic	Both	Climatic
Relative shub cover	(+)	(+)				(-)	(-)			0.52	**64**	21	15
CWM PCA 1	(-)	(-)					(+)		(+)	0.55	**64**	14	22
CWM PCA 2							(-)	(-)		0.20	0	0	**100**
FDis PCA 1	(+)	(+)	(-)					(+)		0.42	**76**	17	7
FDis PCA 2		(-)		(+)	(-)	(-)	(+)			0.36	39	0	**61**

Aritidity= – aridity index.

The PCA of the CWMs of the studied 12 plant traits segregated two main PCA components, which accounted for 57% of the total variance found in the data (Figure 6.2A). The first component, hereafter called 'CWM-PCA1', explained 46% of the variance and described a gradient of RSC. Communities with lower RSC showed a higher proportion of annuals (graminoids and rosettes), more anemochory dispersal and higher SLA; communities with higher RSC showed higher plant height and maximum root depth, more perennial species and more barochory dispersal. This was shown by the strong correlations found between CWM values of these traits and the CWM-PCA1 (Figure 6.2A, Table 6.3). The second component, hereafter called 'CWM-PCA2', explained 10% of the variance. The traits more strongly correlated with CWM-PCA2 were flowering traits (onset and duration), growth-form (erect and prostrate), dispersal strategy (ectozoochory) and N-fixing ability (Fig. 6.2A, Table 6.3).

Community FDis values were explained by the two main PCA components, accounting for 62% of the total variance found in the data (Fig. 6.2B). The first component, hereafter 'FDis-PCA1', accounted for 42% of the variance and separated communities based on life-cycle, root depth, height and SLA traits, which showed the strongest correlations with this axis (Figure 6.2B, Table 6.3). The second component, hereafter 'FDis-PCA2', explained 20% of the variance and segregated communities according to flowering traits (onset and duration), seed mass and growth-form, all positively correlated with this axis (Figure 6.2B, Table 6.3). The first components of both PCAs, i.e. CWM-PCA1 and FDis-PCA1, were mostly explained by topo-edaphic factors (64% and 76% of the total variance explained by the models, respectively), while climatic variables had a dominant role explaining the second PCA components, i.e., CWM-PCA2 and FDis-PCA2, namely 100% and 61% of the total variance explained by each model, respectively (Table 6.2).

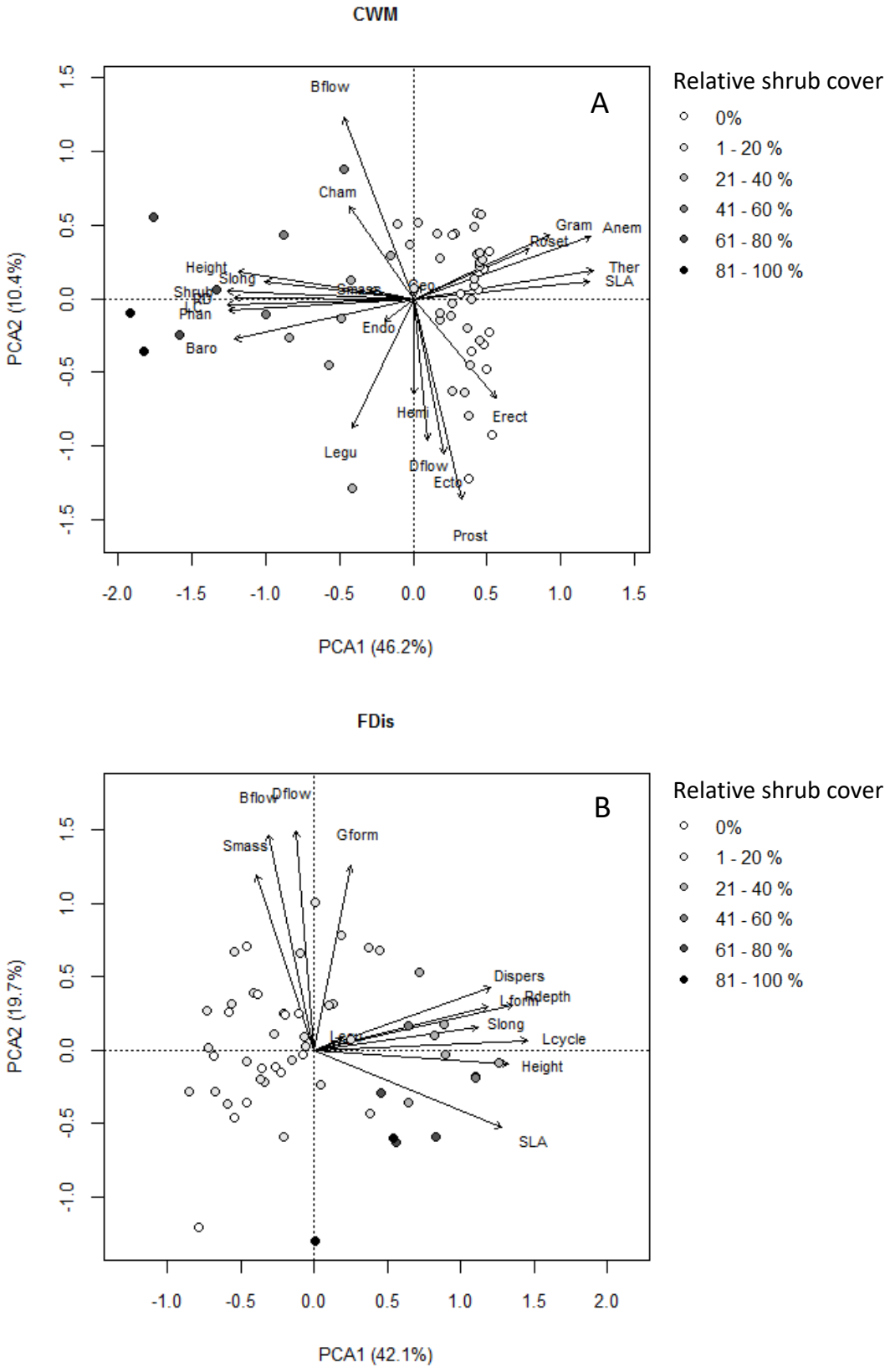

Figure 6.2. Principal component analysis (PCA) of A) community-weighted mean (CWM) and B) functional dispersion (FDis) trait values. Vectors represent traits described in Table S4. Abbreviations: Ther = therophyte; Phan = phanerophyte; Cham = chamaephyte; Gram = graminoid; Roset = rosette; Erect = erect; SLA = specific leaf area; Anem = anemochory; Baro =

barochory; Height = height; Rdepht = root depth; Smass = seed mass; Slong = seed persistence; Dispers = dispersal strategy; Lcycle = life cycle; Bflow = onset of flowering; Dflow = duration of flowering. Study sites are represented by points in a gray scale indicating the class of relative shrub cover (%) at each site (see legend). See Supporting Information Table S5 for correlations among CWM values and Table S6 for correlations among FDis values.

Table 6.3. Pearson correlation coefficients between community-weighted-means (CWM) and the two first components of the respective principal component analysis (CWM-PCA1 and CWM-PCA2); and between functional dispersion (FDis) and the two first components of the respective principal component analysis (FDis-PCA1 and FDis-PCA2). * P<0.05; **P<0.01; ***P<0.001.

Traits	Category	CWM		FDis	
		PCA1	PCA2	PCA1	PCA2
Life cycle		**-0.99*** **	-0.02	**0.95*** **	0.04
Life-form	Therophyte	**0.96*** **	0.10	**0.78*** **	0.16
	Hemicryptoph.	0.01	**-0.35** **		
	Geophyte	0.04	0.05		
	Chamaephyte	**-0.34***	**0.34***		
	Phanerophyte	**-0.98*** **	-0.04		
Growth-form	Bulb	0.04	0.05	0.16	**0.68*** **
	Erect	**0.44** **	**-0.37** **		
	Graminoid	**0.73*** **	0.24		
	Prostrate	0.26	**-0.74*** **		
	Rosette	**0.62*** **	0.19		
	Shrub	**-0.99*** **	0.03		
Max. height		**-0.94*** **	0.10	**0.87*** **	-0.05
SLA		**0.94*** **	0.06	**0.84*** **	**-0.28***
Onset flower.		**-0.37** **	**0.67*** **	-0.20	**0.79*** **
Duration flower.		0.08	**-0.52*** **	-0.08	**0.81*** **
Dispers. strategy	Anemochory	**0.95*** **	0.23	**0.79*** **	0.23
	Barochory	**-0.95*** **	-0.15		
	Ectozoochory	0.16	**-0.57*** **		
	Endozoochory	-0.15	-0.08		
Seed mass		-0.24	0.04	-0.26	**0.65*** **
Seed persistence		**-0.79*** **	0.07	**0.73*** **	0.08
N-fixing ability		**-0.32***	**-0.48*** **	0.13	0.04
Max. root depth		**-0.95*** **	0.00	**0.89*** **	0.17

Our a priory SEM model explained 54% of the variation in RSC, which was mainly driven by topo-edaphic factors. Slope standard deviation and soil C:N ratio had a strong direct positive effect on RSC (Figure 6.3). On the contrary, summer precipitation had a moderate negative direct effect on RSC. Changes in RSC largely determined CWM-PCA1 variation, explaining 98% of its variance through a direct negative effect (Figure 6.3), showing that the effects of topo-edaphic variables and of summer precipitation on CWM-PCA1 (Figure S6.2) were mediated by changes in RSC. The CWM-PCA2 was related only with climatic factors, although they were only able to explain 21% of its

variation; summer precipitation and winter temperature had a direct strong and moderate negative effect on CWM-PCA2, respectively (Figure 6.3). Topo-edaphic factors were negatively related to FDis-PCA1, explaining 64% of its variation; about 3% of this effect was direct, through the interaction between slope standard deviation and soil C:N ratio (although individually these factors had a marginal positive effect), and 97% indirectly, via CWM-PCA1 (Figure 6.3). FDis-PCA2 was related to climatic factors and also to CWM-PCA1, which jointly explained 38% of its variation; about 87% of this effects was direct, driven by a marginal positive influence of summer precipitation and by a negative effect of aridity, and 13% was due to an indirect positive effect of CWM-PCA1 (Figure 6.3).

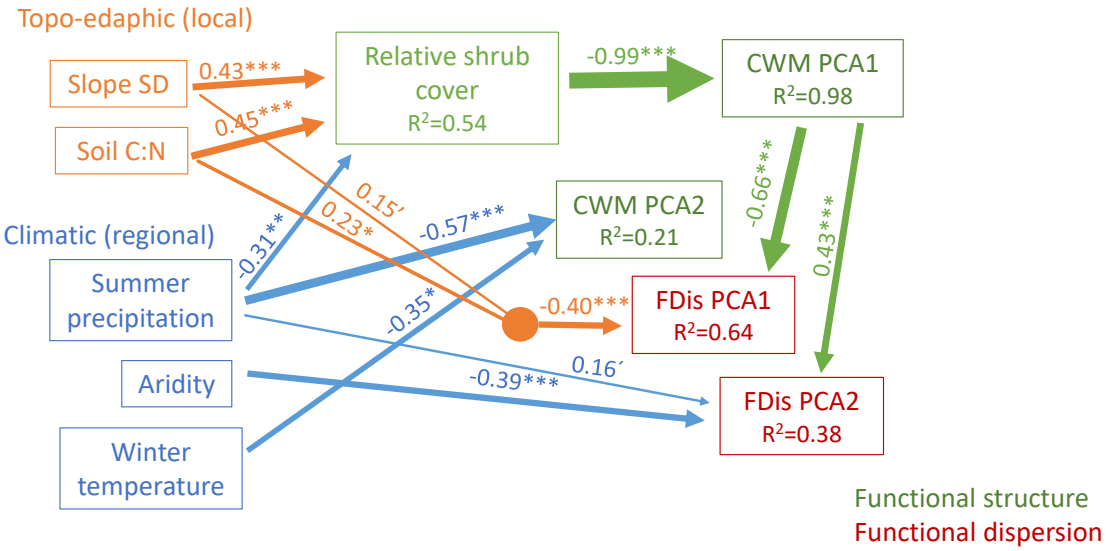

Figure 6.3. Structural equation model to explain relative shrub cover and the main axis of functional specialization of the plant community regarding functional structure (CWM-PCA1 and CWM-PCA2) and functional dispersion (FDis-PCA1 and FDis-PCA2). Overall goodness-of-fit statistics: $\chi^2_{(23)}$ = 26.668, P = 0.270, root mean square error of approximation (RMSEA) = 0.054 (0.00–0.121), comparative fit index (CFI) = 0.989, standardized root mean square residual (SRMR)=0.056. Arrow widths are proportional to the standardized path coefficients, which are presented. The R^2 next to response variables indicates the proportion of variance explained. *P < 0.05; **P < 0.01; ***P < 0.001. Aridity=-Aridity index.

6.5 Discussion

Our results indicate that, under low-intensity and similar land management, local topo-edaphic variables are the most important drivers of relative shrub cover in the studied communities. This highlights the importance of taking local topo-edaphic

variables into account when planning land management at the local scale to deal with shrub encroachment. Shrub encroachment determined the main changes in functional traits' mean and range of the studied communities, particularly of traits differentiating shrubs from herbaceous species, mostly plant size and leaf traits. Climatic drivers played a less prominent role explaining variations in relative shrub cover, as a 'second' environmental filter. We suggest that the effect of precipitation is probably being mediated by the control that local soil properties and topography exert on water (and nutrient) availability for plants. In addition, climatic drivers also directly affected the mean and range of plant traits related to flowering and dispersal strategies.

Environmental drivers of shrub encroachment

Our findings partially match our first hypothesis, as both topo-edaphic and climatic variables influenced shrub encroachment, jointly accounting for 52% of its variation. These results are in accordance with previous works reporting topo-edaphic controls of woody plant cover or biomass, e.g. (Colgan et al., 2012). However, in the communities studied, topo-edaphic factors explained most of the variation in relative shrub cover (64% of the R^2), which increased with slope standard deviation, i.e. towards more rugged relief areas, and with higher soil C:N ratio. In dryland areas, where water limitations often constrain vegetation cover favoring runoff and erosion in steeper areas, topography largely controls water and nutrient flow paths from upslope to lowlands, where they tend to accumulate (Gómez-Plaza et al., 2001). This leads to poorly developed coarser-textured soils on hillslopes, which favor water accumulation at deeper soil depths, favoring deep-rooted species such as shrubs. Conversely, finer-textured soils often found in plain areas, would lead to shallower water profiles thus favoring shallow-rooted species such as most herbaceous species (Sala et al., 1997, McAuliffe, 2003).

Higher soil C:N ratio, which showed no significant correlation with slope standard deviation (Table S3), had also a positive effect on shrub encroachment. The C:N ratio is a proxy for soil biological activity, reflecting the dynamic interaction between slow-modifying soil features (such as particle size distribution and mineralogy) and site variables like microclimate, local hydrology, and vegetation. The C:N ratio is commonly used as an indicator of soil organic carbon turnover, as it influences the rate of decomposition of organic matter: when the C:N ratio is high, the decomposition is slowed by a lack of N (Bot and Benites, 2005). Thus, a higher C:N ratio in shrub encroached areas may indicate a lower soil organic carbon turnover. This may be the result of a soil-

plant-soil interaction, in the sense that, a lower availability of soil nutrients on topsoil (due to low N and/or lower water availability) may favor the colonization by deeper-rooted shrubs; conversely, a higher shrub cover may lead to higher heterogeneity in soil water and nutrients distribution, due to the transport of sediments by wind and water erosion from inter-shrub spaces to 'islands of fertility' surrounding shrub patches, thus promoting further shrub colonization (Schlesinger et al., 1990). Moreover, a higher soil C:N ratio may also be a consequence of litter decomposition of shrubs which has usually a higher C:N ratio and takes more time to decompose than that of herbaceous species (Bot and Benites, 2005). This reciprocal relationship may create a positive feed-back loop promoting shrub encroachment. Relative shrub cover decreased towards areas with increasing summer precipitation across the studied communities. This may be because precipitation during the warmer seasons (summer) usually leads to shallower water profiles, thus favoring shallower-rooted herbaceous species (Sala et al., 1997).

Topo-edaphic and climatic variables jointly explained 52% of the variation in relative shrub cover, suggesting that shrub encroachment may be also influenced by other factors. Low-intensity human activities are known to affect vegetation structure (Castro and Freitas, 2009, Maestre et al., 2009). Low grazing intensity, with occasional plowing and shrub clearing performed in the past along the study area, may have contributed to reduce shrub cover and promote grasslands in areas with lower slope. This is because livestock grazing (particularly cows) occurs preferentially in plain areas (Bailey et al., 1996), probably because the soils are in general more productive and covered mostly by herbaceous species, providing higher quantity and quality of forage. The same applies to mechanical shrub clearing actions, which are easier to perform in flatter areas than in steep slopes. Hence, although a low-intensity land-use was one of the criteria used in sampling site selection, it may have contributed, at least partially, to variations in vegetation structure along the study area, which are not explained by topo-edaphic and climatic variables.

Consequences of shrub encroachment for community functional traits

Changes in relative shrub cover explained most of the variation in both functional structure (82% of the cumulative variance explained by the first two axes of the PCA on CWMs) and functional dispersion (62% of the cumulative variance explained by the first two axes of the PCA on FDis) of the traits analysed. Shrub encroachment was reflected in a strong variation in CWMs of traits differentiating woody taller species with low SLA, most of them persisting during summer, with larger seeds, displaying a resource-

conservative strategy, from herbaceous short-lived species with high SLA and high rates of resource acquisition, that avoid drought by dying before summer (Díaz et al., 2016). These variations in CWMs may be seen as different directions of functional specialization, based on conservation/acquisition trade-offs to deal with drought in water-limited environments (Díaz et al., 2016). Interestingly, functional dispersion of these same traits, particularly of life-cycle, root depth, height and SLA described by the FDis-PCA1, increased with shrub encroachment. This is likely due to the co-occurrence of herbaceous species and shrubs, displaying different resource-use strategies, in areas with higher relative shrub cover, increasing the complexity of vegetation stratification when compared to grasslands devoid of shrubs. The prominent effect of topo-edaphic variables and, to a lesser extent, of climatic factors, in explaining variation along CWM-PCA1 and FDis-PCA1 axes was virtually all mediated indirectly via changes in relative shrub cover.

Other effects of environmental drivers on community functional traits

Although shrub encroachment explained most of the variation in trait mean and range along the study area, we also found a different set of traits described by the second components of PCAs, not so obviously related to changes in shrub cover. The functional structure of these traits (CWM-PCA2) was mostly influenced by summer precipitation and winter temperature, suggesting that climate acts as a second environmental filter on community functional traits. Precipitation and temperature are known to be good predictors of many plant traits (Moles et al., 2014), and can significantly affect the timing of plant phenophases (Peñuelas et al., 2004). Higher summer precipitation and winter temperature favored flowering duration, while flowering onset showed the opposite trend, i.e. plants begun flowering earlier under higher summer precipitation and higher winter temperature. For many species, flowering duration and onset are related in the sense that when species begin flowering earlier in the season, flowering duration tends to be longer (Crimmins et al., 2013). Precipitation during the warmer season may attenuate water limitations and thus enable a longer growth period and flowering duration, particularly for herbaceous species (Crimmins et al., 2013, Ramos et al., 2015), which have shallower root systems and can take better advantage of small rain events than woody species. Similarly, higher winter temperatures may provide more favorable conditions for vegetative growth onset. Previous studies reported an earlier onset of flowering triggered by higher mean annual temperatures e.g. (Miller-Rushing and Primack, 2008), supporting our results. More favorable climatic conditions (i.e. lower aridity levels and higher summer precipitation) were also associated with higher FDis of

flowering traits (onset and duration of flowering) and of seed mass. A higher FDis is expected to reflect a higher complementary in resource use between species, suggesting an improvement in ecosystem functioning (Mouillot et al., 2011). Conversely, a lower FDis in drier sites most likely implies a reduction in ecosystem functions, as found by previous studies (Valencia et al., 2015). Additionally, a lower FDis in more arid sites, by narrowing the range of strategies adopted by plants, might reduce the resilience of dryland ecosystems to environmental change, as hypothesized by (Volaire et al., 2014). This is because it would reduce the chances that some species less susceptible to new environmental conditions could survive, and thus maintain similar levels of ecosystem functioning.

More favorable climatic conditions also enhanced the CWM of prostrate and erect growth-forms. However, it seems to be reflecting not a particular advantage of these strategies under such conditions (e.g. prostrate growth-form may be associated to a higher stress-tolerance), as they are inherently contradictory, but instead it suggests a higher diversity of growth-forms. Accordingly, we found higher FDis for growth-form (described by FDis-PCA2) under lower aridity and higher summer precipitation. This can be also the explanation for a higher proportion of ectozoochorous dispersal and N-fixing species, both significantly correlated with CWM-PCA2, found under more favorable climatic conditions. Attenuation of water limitations and more favorable temperatures in winter may allow a higher reproductive success of species with different ecological strategies, enabling their persistence at a higher proportion in the plant community. Legume species, in particular, seem to be highly vulnerable to drought during their reproductive phase, which may shorten the duration of reproductive development, reduce the number of pods or reduce seed weight and the number of seeds per pod (Daryanto et al., 2015).

6.6 Concluding remarks

Most of the scientific evidence of how multiple environmental factors interact to explain shrub encroachment into former grasslands is from outside of the Mediterranean Basin (Roques et al., 2001, D'odorico et al., 2012, Iannone et al., 2015). Since it is recognized as a context-dependent phenomenon e.g. depending on the functional characteristics of the woody and herbaceous species involved (Maestre et al., 2016), its causes need to be better understood and quantified in the Mediterranean Basin context. Our work, by quantifying the relative importance of local and regional environmental

drivers on shrub encroachment and associated shifts on community functional traits, contributes to a better understanding of the ecological mechanisms driving shrub encroachment in Mediterranean dryland areas. Assessing how functional traits change with shrub encroachment is of paramount importance, considering that they largely influence ecosystems processes and associated ecosystem services.

We found that shrub encroachment is largely determined by topo-edaphic factors, and influenced to a much lesser extent by climate, namely by summer precipitation. Our findings imply that management actions aiming at reducing or reversing shrub encroachment and not addressing topo-edaphic factors, like for instance mechanical shrub removal, will most probably fail its goals on the medium-term. We suggest that other strategies involving the manipulations of such factors, for instance, alterations in terrain structure to change water and nutrient flow pathways and accumulation, are more likely to sustainably succeed. Shrub encroachment mediated major changes in the functional structure and dispersion of the plant communities. Nevertheless, climate also affected a set of functional traits not so directly involved in changes from herbaceous to woody dominance. Our results suggest that altered precipitation regimes and an overall increase in aridity forecasted for the Mediterranean Basin may not have a major impact per se on shrub encroachment, but may directly affect other features of the plant community (e.g. shorten flowering duration) and reduce functional diversity for some traits, thus compromising ecosystem functioning. Our findings provide important advances to better predict shrub encroachment at a local scale, now and under a climate change scenario, and to help define more cost-effective and sustainable management actions to deal with shrub encroachment in Mediterranean ecosystems.

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Supporting Information

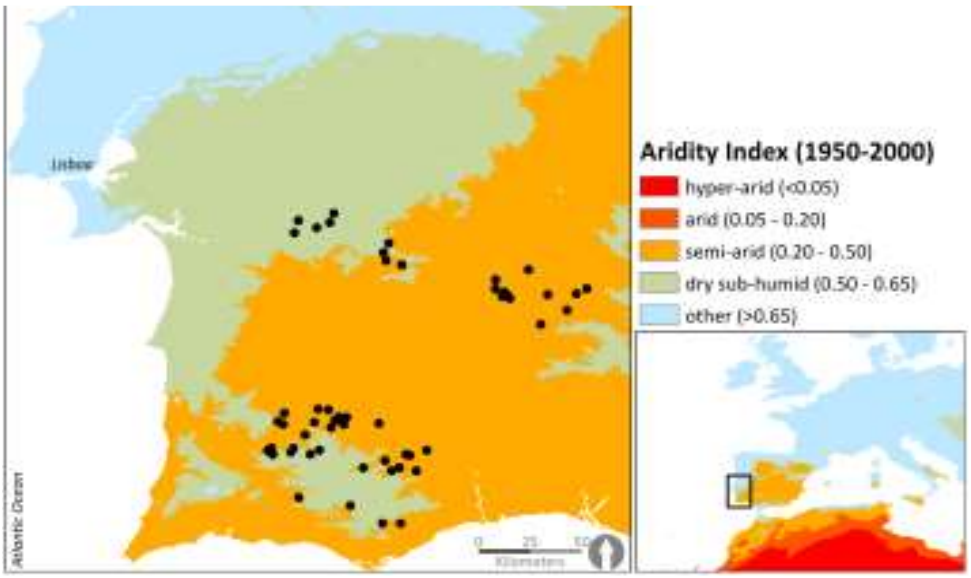

Figure S6.1. Map with the location of the study sites along a climatic gradient.

Table S6.1. Environmental variables considered, their units and range across the study area.

Variables abrev.	Description	Min	Max
Topographic			
Slope (º)	Slope at the centroid of the sampling site	0.81	25.45
Slope SD (º)	Standard deviation of the slope within a 250 m buffer around the centroid of the sampling site, based on digital elevation models, 10 m resolution	1.16	14.69
Topographic wetness index (TWI)	Ln [(upslope area)/local slope], used to quantify topographic control on hydrological processes	6.32	14.44
Climatic			
Aridity index	Ratio of mean annual precipitation to annual potential evapotranspiration, for the period 1950–2000	0.42	0.56
bio 1	Annual mean temperature (ºC)	15.8	17.0
bio 2	Mean diurnal range (Mean of monthly (max temp - min temp))	9.4	11.1
bio 3	Isothermality (Bio2/Bio7) (* 100)	39	43
bio 4	Temperature Seasonality (standard deviation *100)	43.5	58.0
bio 5	Max temperature of warmest month	28.8	32.8
bio 6	Min temperature of coldest month	4.8	6.9
bio 7	Temperature annual range (Bio5-Bio6)	22.2	28
bio 8	Mean temperature of wettest quarter	10.2	12.1
bio 9	Mean temperature of driest quarter	21.5	24.2
bio 10	Mean temperature of warmest quarter	22.00	24.4
bio 11/Winter temp.	Mean temperature of coldest quarter	9.4	11.3
bio 12	Annual precipitation (mm)	520	634
bio 13	Precipitation of wettest month	72	93
bio 14	Precipitation of driest month	1	4
bio 15	Precipitation seasonality (coef. of variation)	56	68
bio 16	Precipitation of wettest quarter	211	265
bio 17/Summer precip.	Precipitation of driest quarter	17	34
bio 18	Precipitation of warmest quarter	20	34
bio 19	Precipitation of coldest quarter	200	262
Edaphic			
Sand	Soil sand content (%)	17.9	71.4
Clay	Soil clay content (%)	8.5	35.3
Silt	Soil silt content (%)	18.1	59.5
SOM	Soil organic matter content (%)	2.3	15.6
Soil N	Soil N content (%)	0.08	0.56
Soil C:N	Soil carbon:nitrogen ratio	6.8	18.1

Table S6.2. Pearson correlation coefficients between the different climatic variables (for variables description see Table S1). Significant correlations ($p<0.05$), as well as variables used in models, are highlighted in bold.

Climatic	**aridity**	bio_1	bio_2	bio_3	bio_4	bio_5	bio_6	bio_7	bio_8	bio_9	bio_10	bio_11	bio_12	bio_13	bio_14	bio_15	bio_16	**bio_17**	bio_18	bio_19
aridity	**1.00**																			
bio_1	**-0.79**	**1.00**																		
bio_2	**-0.75**	**0.60**	**1.00**																	
bio_3	**0.62**	**-0.69**	**-0.75**	**1.00**																
bio_4	**-0.74**	**0.71**	**0.93**	**-0.93**	**1.00**															
bio_5	**-0.74**	**0.76**	**0.95**	**-0.87**	**0.97**	**1.00**														
bio_6	**0.65**	**-0.32**	**-0.81**	**0.75**	**-0.84**	**-0.73**	**1.00**													
bio_7	**-0.76**	**0.68**	**0.97**	**-0.88**	**0.99**	**0.98**	**-0.85**	**1.00**												
bio_8	0.25	-0.19	**-0.70**	**0.73**	**-0.78**	**-0.70**	**0.78**	**-0.76**	**1.00**											
bio_9	**-0.79**	**0.84**	**0.89**	**-0.90**	**0.97**	**0.98**	**-0.71**	**0.95**	**-0.65**	**1.00**										
bio_10	**-0.81**	**0.87**	**0.88**	**-0.89**	**0.96**	**0.97**	**-0.69**	**0.94**	**-0.61**	**0.99**	**1.00**									
bio_11	**0.43**	-0.25	**-0.81**	**0.79**	**-0.85**	**-0.76**	**0.92**	**-0.86**	**0.93**	**-0.71**	**-0.68**	**1.00**								
bio_12	**0.91**	**-0.66**	**-0.42**	**0.35**	**-0.43**	**-0.41**	**0.43**	**-0.44**	-0.08	**-0.50**	**-0.54**	0.12	**1.00**							
bio_13	**0.89**	**-0.88**	**-0.87**	**0.83**	**-0.92**	**-0.94**	**0.67**	**-0.91**	**0.51**	**-0.97**	**-0.97**	**0.61**	**0.66**	**1.00**						
bio_14	-0.21	**0.39**	**0.69**	**-0.69**	**0.74**	**0.76**	**-0.48**	**0.73**	**-0.80**	**0.70**	**0.67**	**-0.72**	0.17	**-0.57**	**1.00**					
bio_15	**0.48**	**-0.63**	**-0.84**	**0.84**	**-0.91**	**-0.93**	**0.61**	**-0.89**	**0.79**	**-0.90**	**-0.88**	**0.77**	0.11	**0.80**	**-0.90**	**1.00**				
bio_16	**0.92**	**-0.87**	**-0.87**	**0.83**	**-0.92**	**-0.93**	**0.71**	**-0.92**	**0.51**	**-0.96**	**-0.97**	**0.63**	**0.70**	**0.99**	**-0.54**	**0.78**	**1.00**			
bio_17	0.05	0.22	**0.52**	**-0.54**	**0.57**	**0.61**	**-0.28**	**0.55**	**-0.73**	**0.53**	**0.49**	**-0.60**	**0.44**	**-0.37**	**0.92**	**-0.83**	**-0.32**	**1.00**		
bio_18	-0.17	**0.43**	**0.64**	**-0.75**	**0.76**	**0.76**	**-0.47**	**0.72**	**-0.81**	**0.72**	**0.69**	**-0.72**	0.20	**-0.57**	**0.94**	**-0.93**	**-0.54**	**0.94**	**1.00**	
bio_19	**0.96**	**-0.85**	**-0.79**	**0.78**	**-0.86**	**-0.84**	**0.72**	**-0.85**	**0.42**	**-0.90**	**-0.92**	**0.57**	**0.82**	**0.96**	**-0.38**	**0.64**	**0.98**	-0.13	**-0.38**	**1.00**

Table S6.3. Pearson correlation coefficients between aridity and topographic and edaphic variables. Significant correlations (p<0.05), as well as variables used in models, are highlighted in bold.

Variables	**aridity**	**SD slope**	slope	TWI	SOM	soil N	**soil C/N**	clay	silt	sand
aridity	1.00									
SD slope	0.16	1.00								
slope	0.05	**0.72**	1.00							
TWI	0.10	**-0.48**	**-0.54**	1.00						
SOM	-0.25	0.06	0.07	-0.14	1.00					
soil N	**-0.36**	-0.13	-0.06	-0.01	**0.81**	1.00				
soil C:N	0.13	0.02	0.14	-0.04	**0.51**	**0.38**	1.00			
clay	**-0.66**	**-0.27**	-0.16	0.05	0.26	**0.30**	0.13	1.00		
silt	**-0.53**	-0.15	-0.01	-0.06	**0.47**	**0.53**	0.17	**0.57**	1.00	
sand	**0.68**	0.24	0.12	-0.01	**-0.47**	**-0.52**	-0.19	**-0.90**	**-0.83**	1.00

Table S6.4. Description of the plant functional traits studied. The sources used to obtain trait information are indicated by superscript numbers: [1]directly observed or measured in the field following standard protocols (Pérez-Harguindeguy et al. 2013); [2]derived from various bibliographic sources (Supplementary material Appendix 1); [3]obtained both through direct measurement in the field and from bibliographic sources.

Type	Trait	Type	Categories/units
Vegetative	Life cycle[3]	ordinal	Annual (0), biennial (0.5), perennial (1)
	Life-form[3]	categorical	Therophyte (annual and facultative biennial), hemicryptophyte, geophyte, chamaephyte, phanerophyte
	Growth-form[1]	categorical	Bulb, erect, graminoid, prostrate, rosette, shrub
	Max. height[1]	continuous	cm
Leaf	SLA[3]	continuous	mm^2/mg
Reproductive	Onset of flowering[2]	semi-cont.	Initial month
	Duration of flowering[2]	semi-cont.	Number of months
Regenerative	Dispersal strategy[3]	categorical	Anemochory, barochory, ectozoochory, endozoochory
	Seed mass[2]	continuous	mg
	Seed persistence[2]	ordinal	Transient (<1 year; 0), short-term persistence (1<years<5; 0.5), long-term persistence (> 5 years; 1)
Below-ground	Nutrient uptake[2]	binary	Has N-fixing ability or not

Type	Trait	Type	Categories/units
	Max. root depth[2]	continuous	geometric mean of rooting depth (m) for shrubs, semi-shrubs, perennial grasses, perennial forbs, annuals and succulents in water-limited ecosystems with winter seasonality of precipitation (Schenk and Jackson 2002)

Table S6.5. Pearson correlation coefficients among community-weighted-mean trait values for different traits. Abbreviations: Ther = therophyte; Hemi = hemicryptophyte; Geo = geophyte; Cham = chamaephyte; Phan = phanerophyte; Erect = erect; Gram = graminoid; Prost = prostrate; Roset = rosette; Shrub = shrub; Height = height; SLA = specific leaf area; Bflow = onset of flowering; Dflow = duration of flowering; Anem = anemochory; Baro = barochory; Ecto = ectozoochory; Endo = endozoochory; Smass = seed mass; Slong = seed persistence; Legu = N-fixing ability; RD = root depth.

CWM			Lform					Gform									Dispers							
		Lcycle	Ther	Hemi	Geo	Cham	Phan	Erect	Gram	Prost	Roset	Shrub	Height	SLA	Bflow	Dflow	Anem	Baro	Ecto	Endo	Smass	Slong	Legu	RD
Lcycle		1.00																						
Lform	Ther	**-0.98**	1.00																					
	Hemi	0.09	-0.21	1.00																				
	Geo	-0.04	-0.01	0.07	1.00																			
	Cham	0.36	-0.33	-0.11	-0.06	1.00																		
	Phan	**0.97**	-0.94	-0.04	-0.08	0.20	1.00																	
Gform	Erect	-0.44	0.45	0.09	-0.02	-0.21	-0.43	1.00																
	Gram	-0.71	0.69	0.11	0.05	-0.20	-0.73	-0.01	1.00															
	Prost	-0.26	0.18	0.14	0.07	-0.21	-0.26	0.09	0.08	1.00														
	Roset	-0.64	0.62	-0.14	-0.03	-0.27	-0.60	0.20	0.11	-0.01	1.00													
	Shrub	**0.99**	-0.95	-0.06	-0.09	0.38	0.98	-0.45	-0.73	-0.29	-0.62	1.00												
Height		0.92	-0.85	-0.05	-0.10	0.33	0.92	-0.40	-0.61	-0.36	-0.63	**0.93**	1.00											
SLA		-0.92	0.89	0.01	0.14	-0.13	**-0.96**	0.39	0.76	0.25	0.49	-0.93	-0.87	1.00										
Bflow		0.36	-0.30	-0.07	0.10	0.46	0.30	-0.36	-0.11	-0.45	-0.17	0.37	0.43	-0.25	1.00									
Dflow		-0.04	0.03	0.19	-0.18	-0.06	-0.05	0.61	-0.14	0.09	-0.12	-0.06	-0.05	0.07	-0.46	1.00								
Dispers	Anem	**-0.94**	0.92	-0.04	-0.03	-0.22	-0.94	0.28	0.80	0.07	0.61	-0.93	-0.86	0.91	-0.21	-0.01	1.00							
	Baro	0.94	-0.91	0.04	0.04	0.19	0.95	-0.26	-0.79	-0.19	-0.59	0.93	0.88	-0.92	0.24	0.03	**-0.99**	1.00						
	Ecto	-0.14	0.10	0.15	0.03	-0.12	-0.14	0.00	0.07	0.68	-0.08	-0.16	-0.23	0.13	-0.30	-0.05	0.02	-0.16	1.00					
	Endo	0.14	-0.18	-0.17	-0.06	0.46	0.08	-0.20	-0.13	0.28	-0.13	0.16	0.05	0.00	0.08	-0.12	-0.18	0.06	0.12	1.00				
Smass		0.21	-0.18	-0.16	-0.13	0.18	0.21	-0.43	0.05	0.10	-0.28	0.23	0.25	-0.14	0.02	-0.40	-0.17	0.12	0.20	0.27	1.00			
Slong		0.76	-0.71	-0.21	0.07	0.18	**0.80**	-0.33	-0.54	-0.23	-0.58	0.79	0.71	-0.77	0.25	-0.01	-0.74	0.77	-0.18	-0.02	0.14	1.00		

CWM		Lform					Gform									Dispers							
	Lcycle	Ther	Hemi	Geo	Cham	Phan	Erect	Gram	Prost	Roset	Shrub	Height	SLA	Bflow	Dflow	Anem	Baro	Ecto	Endo	Smass	Slong	Legu	RD
Legu	0.30	-0.34	0.09	-0.02	-0.04	0.31	-0.12	-0.31	0.37	-0.21	0.28	0.28	-0.27	-0.05	0.01	-0.38	0.33	0.09	0.30	0.29	0.03	1.00	
RD	**0.96**	-0.96	0.12	-0.06	0.34	0.94	-0.49	-0.74	-0.29	-0.47	0.95	0.87	-0.90	0.34	-0.11	-0.89	0.89	-0.16	0.15	0.21	0.65	0.35	1.00

Table S6.6. Pearson correlation coefficients among functional dispersion trait values for different traits. Lcycle = life cycle; Lform = life-form; Gform = Growth-form; Height = height; SLA = specific leaf area; Bflow = onset of flowering; Dflow = duration of flowering; Dispers = dispersal strategy; Smass = seed mass; Slong = seed persistence; Legu = N-fixing ability; Rdepht = root depth.

FDis	Lcycle	Lform	Gform	Height	SLA	Bflow	Dflow	Dispers	Smass	Slong	Legu	Rdepth
Lcycle	1.00											
Lform	0.68	1.00										
Gform	0.13	0.26	1.00									
Height	**0.83**	0.57	0.17	1.00								
SLA	**0.82**	0.43	-0.10	0.73	1.00							
Bflow	-0.18	-0.04	0.28	-0.29	-0.36	1.00						
Dflow	-0.02	0.03	0.34	-0.09	-0.26	0.66	1.00					
Dispers	0.74	0.47	0.38	0.67	0.64	0.06	0.06	1.00				
Smass	-0.14	-0.19	0.39	-0.19	-0.30	0.40	0.38	-0.12	1.00			
Slong	0.66	0.52	-0.11	0.49	0.62	0.10	0.08	0.55	-0.16	1.00		
Legu	0.04	-0.04	-0.11	0.00	0.09	0.12	0.00	0.24	-0.02	0.39	1.00	
Rdepth	**0.85**	**0.91**	0.28	0.73	0.60	-0.12	0.05	0.56	-0.09	0.59	-0.01	1.00

Table S6.7. Best-fitting regression models ranked according to their AIC values.

	Topo-edaphic					Climatic				Topo-edaphic x Climatic				
Response variables	Slope SD	Soil C/N	Slope SD x Soil C/N	Sand	soil C/N x Sand	Aridity index	Aridity^2	Summer precip.	Winter temp.	Slope SD x Summer precip.	Adj-R^2	AIC	ΔAIC	wi
Shrubland cover	(+)	(+)				(+)		(-)			0.52	120.1	0	0.54
	(+)	(+)						(-)			0.51	120.5	0.3	0.46
CWM PCA 1	(-)	(-)						(+)		(+)	0.55	116.34	0.0	0.85
	(-)	(-)						(+)			0.52	119.74	3.4	0.15
CWM PCA 2								(-)	(-)		0.20	123.8	0	1.00
FDis PCA 1	(+)	(+)	(-)						(+)		0.42	90.5	0.0	0.53
	(+)	(+)	(-)			(+)					0.40	92.0	1.5	0.25
	(+)	(+)	(-)								0.39	92.2	1.8	0.22
	(+)	(+)							(+)		0.30	99.5	9.1	0.01
	(+)	(+)				(+)					0.28	101.4	11.0	0.00
	(+)	(+)									0.26	101.9	11.4	0.00
FDis PCA 2		(-)		(+)	(-)	(+)		(+)			0.36	96.9	0.0	0.65
		(-)		(+)	(-)	(+)	(+)	(+)			0.34	98.9	2.0	0.24
		(-)				(+)		(+)			0.29	100.4	3.5	0.11

AIC measures the relative goodness of fit of a given model; the lower its value, the more likely the model to be correct. Wi - Akaike weight

Table S6.8. Description of the final regression models obtained.

	Estimate	Std. Error	t-value	p-value	Adj. R^2
Relative shrub cover					0.52
(Intercept)	2.65E-16	9.41E-02	0	1.00000	
Aridity index	1.44E-01	9.75E-02	1.474	0.14681	
Summer precip.	-3.22E-01	1.05E-01	-3.082	0.00337	
Slope SD	4.00E-01	1.04E-01	3.829	0.00037	
Soil C:N	4.34E-01	9.72E-02	4.461	0.00005	
CWM-PCA1					0.55
(Intercept)	1.10E-01	1.03E-01	1.069	0.29024	
Slope SD	-2.95E-01	1.12E-01	-2.627	0.01148	
Summer precip.	3.62E-01	1.02E-01	3.54	0.00089	
Soil C:N	-4.88E-01	9.39E-02	-5.202	0.00000	
Slope SD x Summer precip.	3.04E-01	1.34E-01	2.27	0.02763	
CWM-PCA2					0.2
(Intercept)	1.85E-06	9.90E-02	0	0.99999	
Summer precip.	-4.81E-01	1.25E-01	-3.867	0.00031	
Winter temp.	-3.09E-01	1.25E-01	-2.482	0.01641	
FDis-PCA1					0.42
(Intercept)	3.34E-03	7.15E-02	0.047	0.96295	
Slope SD	3.30E-01	7.73E-02	4.273	0.00009	
Soil C:N	3.25E-01	8.17E-02	3.977	0.00023	
Winter temp.	1.42E-01	7.53E-02	1.882	0.06576	
Slope SD x soil C:N	-1.70E-01	5.10E-02	-3.338	0.00162	
FDis-PCA2					0.36
(Intercept)	-4.95E-02	7.75E-02	-0.639	0.52589	
Aridity index	1.95E-01	1.15E-01	1.699	0.09578	
Summer precip.	3.17E-01	7.80E-02	4.064	0.00018	
Soil C:N	-2.06E-01	8.44E-02	-2.44	0.01844	
Sand	3.83E-02	1.17E-01	0.328	0.74421	
Soil C:N x Sand	-2.64E-01	9.98E-02	-2.649	0.01090	

Chapter 7

Is trait-based ecology being used in dryland ecosystems' restoration?

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7 Is trait-based ecology being used in dryland ecosystems' restoration?

7.1 Abstract

Restoration efforts in the Mediterranean Basin have been changing from a silvicultural to an ecological restoration approach. Yet, to what extent the projects are guided by ecological restoration principles remains largely unknown. To analyse this issue, we built an on-line survey addressed to restoration practitioners. We analysed 36 restoration projects, mostly from drylands (86%). The projects used mainly soil from local sources. The need to comply with legislation was more important as a restoration motive for European Union (EU) than for non-EU countries, while public opinion and health had a greater importance in the latter. Non-EU countries relied more on non-native plant species than EU countries, thus deviating from ecological restoration guidelines. Nursery-grown plants used were mostly of local or regional provenance, whilst seeds were mostly of national provenance. Unexpected restoration results (e.g. inadequate biodiversity) were reported for 50% of the projects and restoration success was never evaluated in 22% of them. Long term evaluation (>6 years) was only performed in 31% of cases, and based primarily on plant diversity and cover. The use of non-native species and species of exogenous provenances may: i) entail the loss of local genetic and functional trait diversity, critical to cope with drought, particularly under the predicted climate change scenarios; and ii) lead to unexpected competition with native species and/or negatively impact local biotic interactions. Absent or inappropriate monitoring may prevent the understanding of restoration trajectories, precluding adaptive management strategies, often crucial to create functional ecosystems able to provide ecosystem services. The overview of ecological restoration projects in the Mediterranean Basin revealed high variability among practices and highlighted the need for improved scientific assistance and information exchange, greater use of native species of local provenance, and more long-term monitoring and evaluation, including functional and ecosystem services' indicators, to improve and spread the practice of ecological restoration.

Keywords: drylands; ecological restoration practice index; native species; provenance; restoration success; survey

7.2 Introduction

The Mediterranean Basin has a long history of human activity which, coupled with its typical climatic regime of cool wet winters and hot dry summers, resulted in plant adaptations to clearing, grazing, fires, and drought (Davis et al., 1996). However, the intensification in land use (e.g. agriculture and grazing), the increase in fire frequencies, as well as urban development (e.g. infrastructure building), led to extensive areas of degraded lands exhibiting low biological productivity and slow ecosystem recovery rates after disturbances or abandonment of land use (Le Houerou, 2000; Zdruli, 2014). High water stress, together with more intense and/or frequent disturbances, often reduce ecosystem resilience, generating a positive feedback which exacerbates land degradation. Most of the Mediterranean Basin territory, about 67%, is occupied by drylands (White and Nackoney, 2003). These water limited areas are classified according to the UNEP aridity index ($0.05<AI<0.65$) (Middleton and Thomas, 1992), and comprise from a higher to a lower aridity level, hyper-arid, arid, semi-arid, and dry sub-humid areas. Drylands are particularly vulnerable to desertification and land degradation (MEA, 2005; Maestre et al., 2012; Reynolds et al., 2007b), which affect the ability of ecosystems to deliver ecosystem services, compromising people's livelihood and well-being (MEA, 2005; Reynolds et al., 2007a). Land degradation may be further aggravated by climate change, which is expected to generate warmer and drier conditions and a higher frequency of extreme events (heat waves, droughts and floods), that are expected to severely impact the Mediterranean Basin (MEA, 2005; IPCC, 2007).

In severely degraded areas with low resilience, restoration is the main means to reverse land degradation, and to restore ecosystems' composition, functioning and sustainability (SER, 2004), thus contributing to improve the welfare of local populations (Suding et al., 2015; Zucca et al., 2013). However, restoration actions are particularly challenging under the stressful conditions found in Mediterranean drylands (Cortina et al., 2011; Vallejo et al., 2012). During the first half of the 20th century, many large restoration projects were conducted across the Mediterranean Basin. Initially, most of them relied on a silvicultural approach, with the introduction of a few fast-growing tree species, and intended to combine forest productivity with hydrological watershed protection, as well as to promote employment in remote rural areas. Hence, despite their contribution to reduce erosion and increase plant cover and productivity, in most cases they led to long-lasting mono-specific tree stands with low diversity (e.g. Cortina et al., 2011). As such, they could not be considered 'ecological restoration' in the strictest sense (Vallejo, 2009), at least without further management to promote biodiversity,

particularly native species. According to the Society for Ecological Restoration (SER), ecological restoration must fulfil clearly stated goals for the target ecosystem: it should become similar to a non-disturbed or 'reference' ecosystem regarding: (i) species diversity, (ii) community structure, (iii) presence of functional groups and of native species, (iv) establishment of biotic fluxes with surrounding areas, (v) self-sustainability, and (vi) resilience to disturbance (SER, 2004; Suding et al., 2015). Moreover, it advocates the integration of scientific and other forms of knowledge into restoration practice, as well as the evaluation of restoration projects outcomes to assess whether the defined objectives are being achieved (SER, 2004). The restoration practice most commonly implemented in the Mediterranean region during the first half of the last century has been progressively replaced by a more ecosystem-based approach, with diversification of plant species, and due consideration given to both soils and fauna preservation. Recent legislation initiatives and environmental policies also played a role in changing the restoration paradigm, particularly in the European Union (EU). Ecological restoration became an essential target of EU vision and strategy for biodiversity, as illustrated by the habitat directive 92/43/EEC and the Convention on Biological Diversity, highlighting the importance of restoring and preserving local biodiversity. This shift in restoration practice has been reported in the Iberian Peninsula (Cortina et al., 2011; Oliveira et al., 2014), but to what extent it has spread to all the Mediterranean Basin remains largely unknown.

Recently, several important efforts have been made to gather information about restoration projects and to make it available, either as shared databases (e.g. REACTION project) or through meta-analysis of published papers (Aronson et al., 2010; Piñeiro et al., 2013). However, such information is often restricted to a region (e.g. the Northern Mediterranean, as in the case of the REACTION project) or the information is likely biased towards 'positive' restoration results. Furthermore, they do not reflect the full range of experience and technical knowledge held by practitioners. Therefore, despite the availability of several published works providing important theoretical reflections and guidelines for dryland restoration (Bainbridge, 2012; Bautista et al., 2009; Vallejo et al., 2012), a comprehensive report and diagnosis of the actual situation from the viewpoint of practitioners could be extremely useful to address a wide range of questions, such as: Does ecological restoration practice integrate scientific knowledge? Are native species being used in restoration projects? Are restoration outcomes evaluated, how, and for how long? Are results as good as expected? Answers to such

questions are vital to assess the current ecological restoration efforts and to improve their efficiency and effectiveness in drylands and, particularly, in Mediterranean areas.

The general aim of this work was to understand whether ecological restoration projects implemented across the Mediterranean Basin follow ecological restoration stated goals, according to SER (SER, 2004). We built an on-line open survey directed towards practitioners. Specifically, we aimed at assessing: 1) if the projects were assisted by scientists; 2) the source of the soils used in restoration (local or non-local); 3) the type of species used (native or non-native) and their provenance (local or other); and 4) if restoration success was evaluated, when, and how (which success indicators were used). We hypothesized that, despite the recent environmental policies and increasing scientific knowledge on ecological restoration, these are still not fully incorporated in restoration practice, namely in the Mediterranean Basin. For each restoration issue assessed we tested the following specific hypotheses: (i) projects differ in the extent to which they follow ecological restoration recommendations; and (ii) restoration practice is influenced by project location (within the EU or not), aridity level and cause of degradation. We thus expected to obtain a comprehensive overview of the current practice of ecological restoration projects implemented in this vulnerable region, in order to critically analyse to what extent they fulfil ecological restoration principles which will allow us to draw lessons to improve ecological restoration practice.

7.3 Methods

Questionnaire contents and dissemination

We built an open on-line survey about ecological restoration of drylands focused on practice. It addressed many aspects of restoration such as context and motivation, planning, implementation and maintenance, species selection, monitoring and success evaluation, and costs and benefits. The questionnaire was in English and consisted mostly of multiple-choice questions (with a free text option), including open-end and dichotomous (yes/no) questions (Appendix A1). The survey was sent out by e-mail and was also advertised on specific sites, with a link to the questionnaire form. Taking advantage of COST Action ES1104 ('Arid lands restoration and combat of desertification: setting up a drylands and desert restoration hub') contact network, we selected as wide a range as possible of professionals involved in ecological restoration of drylands, including practitioners from private companies and associations, governmental

administrations, universities and research institutes, and thematic networks already established (e.g. the International Society for Ecological Restoration, DesertNet International, United Nations Convention to Combat Desertification and the European Society for Soil Conservation). The questionnaire was sent to 1431 contacts and 148 of them were returned. The information from the completed questionnaires was then collected and analysed. Here, we focus on the questionnaires from ecological restoration projects implemented in the Mediterranean Basin (n=36), which are mostly located in dryland areas (n=31), and a few in non-dryland areas (n=5).

Data analysis

The results are presented either as the relative proportion of the restoration projects addressed by the survey displaying a particular answer, or as the 'number of paired answers'. For multiple-choice answers, the sum of the relative proportions calculated for each option often exceeds 100% because the options are not mutually exclusive. The 'number of paired answers' corresponds to the number of times a certain pair of options is chosen simultaneously as a response to two distinct multiple choice questions. Therefore, the number of paired answers reflects the counting of options' combinations, and their sum may surpass the total number of projects addressed by the survey. To integrate the information collected from several questions and classify the projects in terms of compliance with ecological restoration principles, we calculated an ecological restoration practice index. The index was based on six restoration issues addressed in the questionnaire clearly associated with ecological restoration recommendations (SER, 2004), and whose answers were independent of the respondent subjective opinion. The issues addressed were: (i) scientific assistance to restoration projects, as it is important to integrate the current knowledge about ecosystems' complexity and functioning in restoration practice; (ii) the source of the soil used in restoration, as it contains propagules thus playing a crucial role in the evolution and sustainability of the restored ecosystem; (iii) the type of species used, considering the preferential use of native species; (iv) the provenance of species propagules, considering the desirable conservation of species' genetic and functional trait diversity; (v) restoration success evaluation, as the only way to learn from experience, adapt management strategies and optimize restoration practice; and vi) restoration success indicators used, preferably linked to ecosystem functioning and thus to its sustainability. We attributed a score to each answer received (from 0 to 2), and the higher the score, the higher the agreement between the practice and ecological restoration principles. The

final index value corresponds to the average of the scores we attributed to the six issues, as follows:

(i) scientific assistance to the project (before, during, and after the restoration project = 2; only in one or two of such occasions = 1; never = 0);
(ii) source of the soil used in restoration activities (only local = 2; local + other = 1; only non-local = 0);
(iii) type of species used (only native = 2; native + non-native = 1; only non-native = 0);
(iv) plant propagules' provenance (local = 2; local + other = 1; only national or international = 0);´
(v) duration of monitoring for success evaluation (> 5 years = 2; 1 - 5 years = 1; never = 0);
(vi) indicator(s) used to evaluate restoration success (based also on functional indicators, e.g. soil organic matter, litter decomposition rate, soil microbiological diversity = 2; based only on species diversity, e.g. multi-taxa = 1; based only on diversity, vitality or cover of plant species = 0).

For data analysis, we used an estimate of the aridity index (the ratio of mean annual precipitation to annual potential evapotranspiration) for the period 1950-2000 for each restoration site. Aridity was retrieved from a global database (Trabucco and Zomer, 2009), based on the approximate geographic location of each project.

Statistical Analysis

We built generalized linear models (GZLM) using binomial distribution and the logit link function to test the importance of aridity (aridity index), project location (countries inside and outside EU), and cause of degradation (overgrazing/agriculture, infrastructure/industry and fire) for all the studied variables, coding each category of the response variables as dummy variables (binary, 0 or 1). We used general linear models (GLM) to test the importance of the same explanatory variables (aridity, location and degradation cause) for the ecological restoration practice index. By stratifying *a posteriori* the answers according to explanatory variables of interest (e.g. geographic location), we got unbalanced sample sizes which reduced the power of the statistical tests in some cases. Whenever the sample size was too low (e.g. n<5), the interpretation of the results took this into consideration. All the analyses were performed under R statistical environment (R Core Team, 2015).

7.4 Results

Characterization of restoration projects and of respondents

Here, we analyse the answers from 36 restoration projects implemented in terrestrial ecosystems distributed over 16 countries mostly from the Mediterranean Basin. We have included two projects not strictly belonging to this region (Georgia and Armenia), because of their geographic proximity and climatic similarity; 23 projects were from European Union (EU) countries, while 13 were from non-EU countries (nEU) (Fig. 7.1, Table 7.1). Projects implemented in drylands were mostly from semi-arid areas (n=20), followed by dry sub-humid (n=7) and arid areas (n=4), whereas 5 projects were from non-dryland areas, i.e. with an aridity index > 0.65 (Middleton and Thomas, 1992) (Table 7.1).

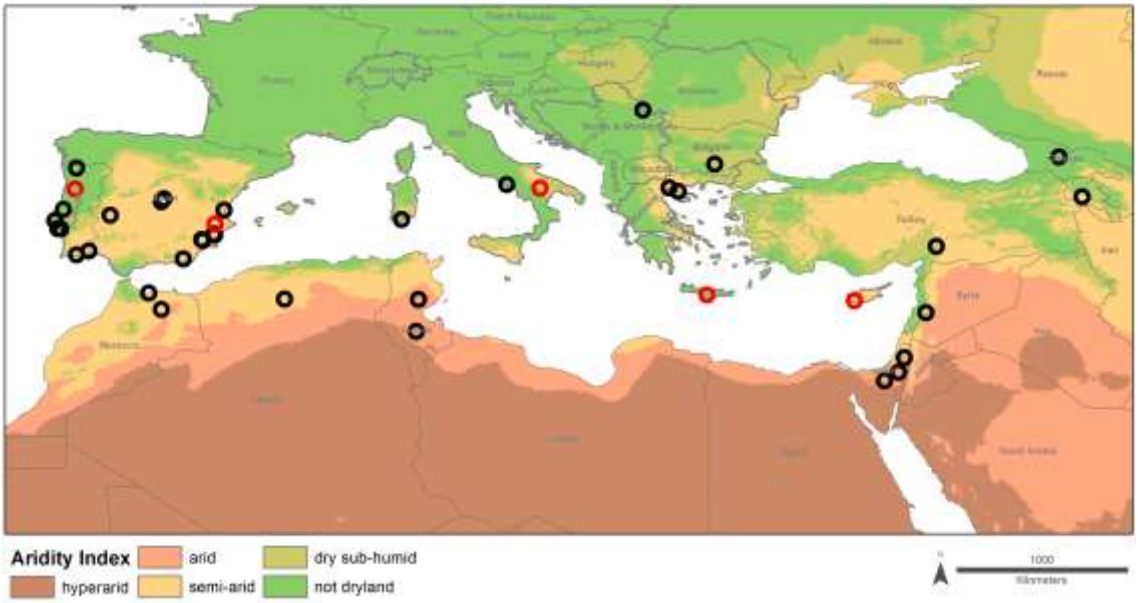

Figure 7.1. Geographical distribution and aridity index of the surveyed ecological restoration projects. The red circles indicate five sites belonging to the same project (one questionnaire). More information about each project can be found in Table S7.1.

Most projects encompassed more than one vegetation type, e.g. shrublands intermingled with perennial and annual grasslands (Table 7.1). Overall, the most represented habitats were shrublands (56%), annual grasslands (33%), and forest/woodlands (31% of the projects). Perennial grasslands were represented in 19% of the projects, savannas and riparian habitats in 6% each, and dunes in 8% (Table 7.1, Fig. S7.1). The implementation areas of the projects varied considerably, ranging from

0.8 ha to 48,124 ha, and no significant association was found between the size of the project area and any of the restoration issues addressed (data not shown).

Table 7.1. Brief description of the surveyed restoration projects: context, country, classification according to the United Nations Environmental Programme (UNEP) aridity index, and habitat type (FW - Forest/Woodland; SR - Shrubland; SV - Savanna; AG - Annual grassland; PG - Perennial grassland; DN - Dunes; RP - Riparian).

Project context	Country	Aridity level	Habitat type						
			FW	SR	SV	AG	PG	DN	RP
Sand dune restoration	Israel	Arid						X	
Overgrazed lands	Israel	Arid				X			
Overgrazed agropastoral systems	Tunisia	Arid		X		X			
Deforested/overgrazed lands	Tunisia	Arid	X	X		X	X		
Deforested lands	Algeria	Semi-arid				X		X	
Quarry restoration	Greece	Semi-arid		X			X		
Quarry restoration	Greece	Semi-arid	X						
Overgrazed/burned lands	Italy	Semi-arid	X						
Overgrazed agropastoral systems	Morocco	Semi-arid		X					
Deforested/agricultural lands	Morocco	Semi-arid	X						
Deforested lands	Palestine	Semi-arid		X		X			
Agricultural/burned lands	Portugal	Semi-arid		X					
Quarry restoration	Portugal	Semi-arid			X				
Quarry restoration	Spain	Semi-arid		X					
Agricultural/burned lands	Spain	Semi-arid		X					
Overgrazed lands	Spain	Semi-arid			X				
Quarry restoration	Spain	Semi-arid	X	X		X	X		
Degraded orchards and vineyards	Spain	Semi-arid		X					
Deforested/agricultural lands	Spain	Semi-arid		X					
Overgrazed/burned lands	Spain	Semi-arid	X	X					
Agricultural lands	Spain	Semi-arid		X		X	X		
Burned lands	Spain	Semi-arid		X					
Deforested/overgrazed lands	Spain	Semi-arid		X					
Burned lands	*Several*[a]	Semi-arid	X						
Overgrazed pastures	Armenia	Dry subhumid				X	X		
Burned degraded lands	Bulgaria	Dry subhumid	X						
Overgrazed agropastoral systems	Lebanon	Dry subhumid				X			
Pit mine restoration	Portugal	Dry subhumid	X						
Quarry restoration	Portugal	Dry subhumid		X					
Burned/eroded lands	Serbia	Dry subhumid	X				X	X	X
Deforested/overgrazed lands	Turkey	Dry subhumid	X	X		X			
Agricultural/overgrazed lands	Georgia	Not dryland				X			
Deforested/eroded lands	Italy	Not dryland		X					
Deforested/eroded lands	Portugal	Not dryland		X					
Deforested/eroded lands	Portugal	Not dryland							X
Quarry restoration	Portugal	Not dryland		X		X	X		

[a] Portugal, Spain, Italy, Greece, Cyprus.

In general, the main causes of land degradation were overgrazing (33%), infrastructure development (33%) and intensive agriculture (31%), whereas industrial activities (i.e. quarries and pit mines) and fire were indicated each in 22% of the projects. Deforestation and climatic constraints (e.g. drought), in many cases associated with the former degradation causes, were noted in 33% and 17% of the projects, respectively (Fig. 7.2). No significant association was found between the degradation causes and the aridity level, or with the location of projects (Fig. 7.2, Table S7.2).

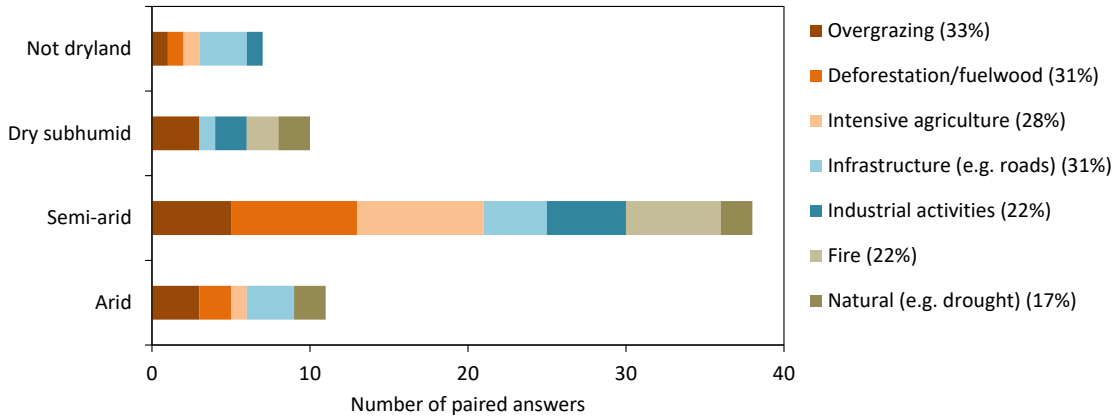

Figure 7.2. Number of times each degradation cause was reported for each aridity level (number of paired answers). The overall relative proportion of restoration projects referring to each degradation cause is displayed within brackets.

Overall, most restoration projects (39%) were motivated by the need to comply with general legislation. It was relatively more important in the EU than in nEU countries ($p<0.01$), especially to regulate the rehabilitation of areas affected by industrial activities and associated deforestation, along with the initiative of the companies responsible for those activities (22% of all projects) (Fig. 7.3, Table S7.2). Many restoration projects (33%) were also fostered by governmental initiatives such as central or regional administrations or rural support programs. The pressure from public opinion (22%) and public health issues (11%) had greater relative importance in nEU countries than in EU countries ($p<0.01$). A few projects were motivated by specific and usually more restrictive legislation regulating restoration activities in protected areas (e.g. natural parks) (14%) (Fig. 7.3).

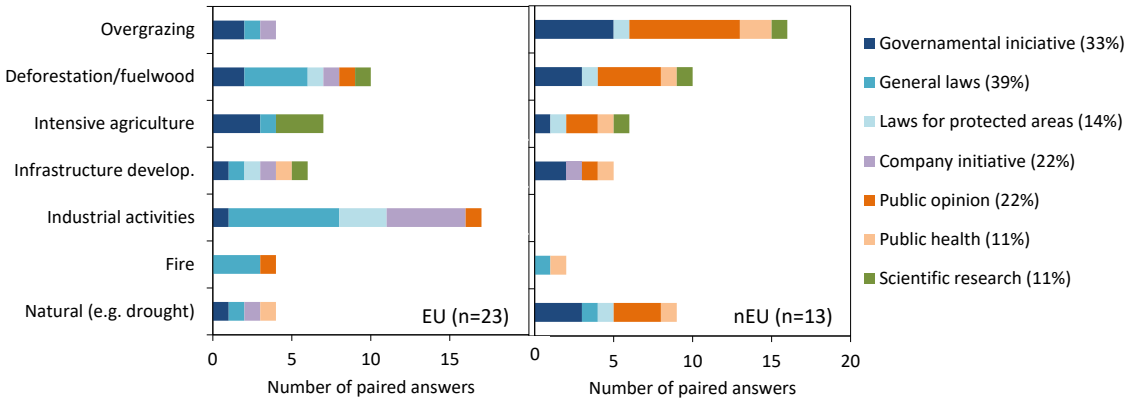

Figure 7.3. Number of times each restoration motivation was reported for each degradation cause (number of paired answers), for restoration projects from European Union countries (EU) and from other countries (nEU). The relative overall proportion of projects referring to each motivation is displayed within brackets.

Most of the respondents worked at universities or research institutes (n=26) while the remaining worked in the private sector (n=6), governmental institutions (n=3) or non-governmental organizations (n=1) (data not shown). The respondents were chiefly researchers or scientific consultants (64%), most of whom were ecologists (33%), soil scientists (22%) or forest engineers or agronomists (19%) (Table 7.2). Restoration activities were primarily planned by scientists/researchers (39% of the projects), followed by Conservation or Forestry State Institutes technicians (16%), and by employees of the involved local company (12%). The implementation of restoration activities was mainly done by the latter (25% of the projects) (Table S7.3).

Soil source, species selection, provenance of propagules and revegetation techniques

Only 8% of all projects used non-local topsoil for restoration; this occurred only in areas where land degradation was due to infrastructure development or industrial activities. The majority of the projects utilised original topsoil already eroded or disturbed (75%), alone or in combination with undisturbed local soil (33%), or with the reintroduction of local topsoil before plant introduction (22%) (Fig. 7.4A). No significant differences were found in the soil source used in restoration projects between different aridity levels or locations (Table S7.2).

Table 7.2. Characterization of the survey respondents regarding their academic background and role in the restoration project.

Background	Company representative	Project manager or coordinator	Researcher/ consultant	Total percentage
Ecologist		1	11	*33.3%*
Soil engineer/scientist		3	5	*22.2%*
Forest engineer/agronomist	1	3	3	*19.4%*
Geoscientist	1		2	*8.3%*
Environmental-social scientist		1	2	*8.3%*
Industrial technician	2			*5.6%*
Biochemist		1		*2.8%*
Total percentage	11.1%	25.0%	63.9%	

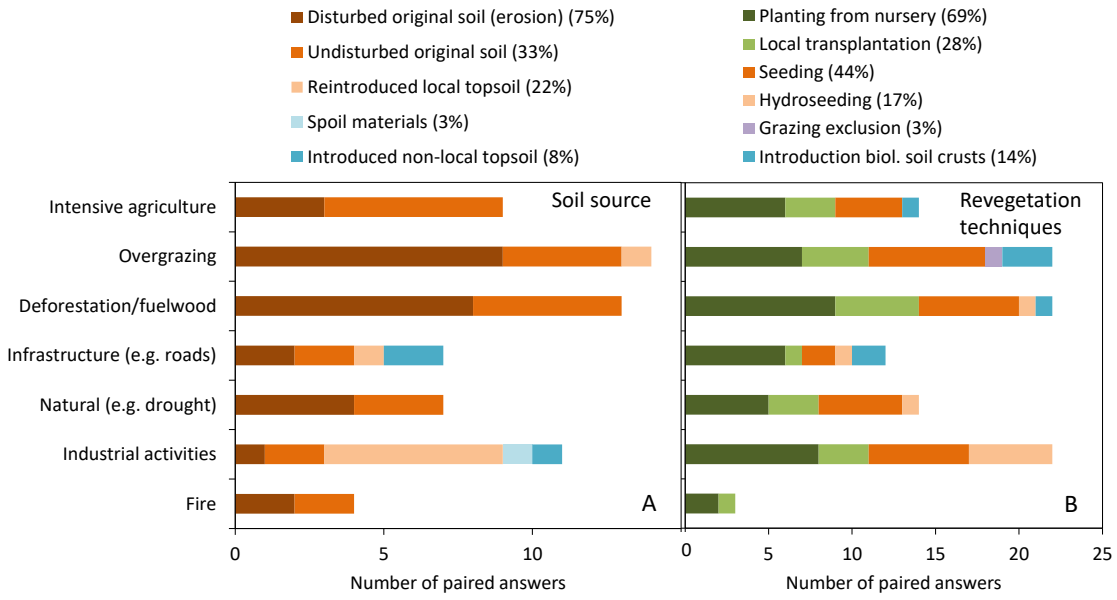

Figure 7.4. Number of times each soil source (A) and revegetation technique (B) was reported for each degradation cause (number of paired answers). The relative overall proportion of restoration projects referring to each soil source (A) and revegetation technique (B) is displayed within brackets.

The majority of the projects surveyed relied on the introduction of plant species (89%, data not shown). Revegetation was made with nursery-grown seedlings in 69% of the projects, whereas 44% included seeding and 28% used local transplantations, regardless of the degradation cause (Fig. 7.4B). Hydroseeding (17%) was carried out exclusively in restoration actions following industrial activities (mining) or infrastructure development, in some cases associated with deforestation and drought. The introduction (inoculation) of biological soil crusts (BSC) was used in 14% of the projects; although no

statistical significant differences were found, probably due to unbalanced and low number of samples in each case, the use of BSC was associated with degradation driven by infrastructure development (and consequent deforestation), overgrazing or intensive agriculture (Table S7.2). Grazing exclusion was used as a restoration strategy in 3% of the projects (Fig. 7.4B). A higher percentage of projects used exclusively native species in EU countries than in nEU countries, both in terms of seedlings (48% and 20%, respectively; $p<0.05$) and seeds (47% and 22%, respectively; $p<0.05$) (Fig. 7.5A-B, Table S7.2). Conversely, more restoration projects used mainly non-native plant species in nEU countries when compared to EU countries, in terms of seedlings (10% and 40%, respectively; $p<0.05$) and seeds (5% and 44%, respectively; $p<0.05$) (Fig. 7.5A-B, Table S7.2). No relationship was found between the nativeness of the species used in restoration and different aridity levels or degradation causes (Table S7.2). The main reasons pointed out for the use of non-native species, which happened in 47% of the projects (n=17), were a usually higher growth rate relative to native species (65%), and a greater commercial availability (47%) at a lower price (24%). Nurse-effects and aesthetic values were also reported each for 18% of the projects using non-native species (Fig. S7.2).

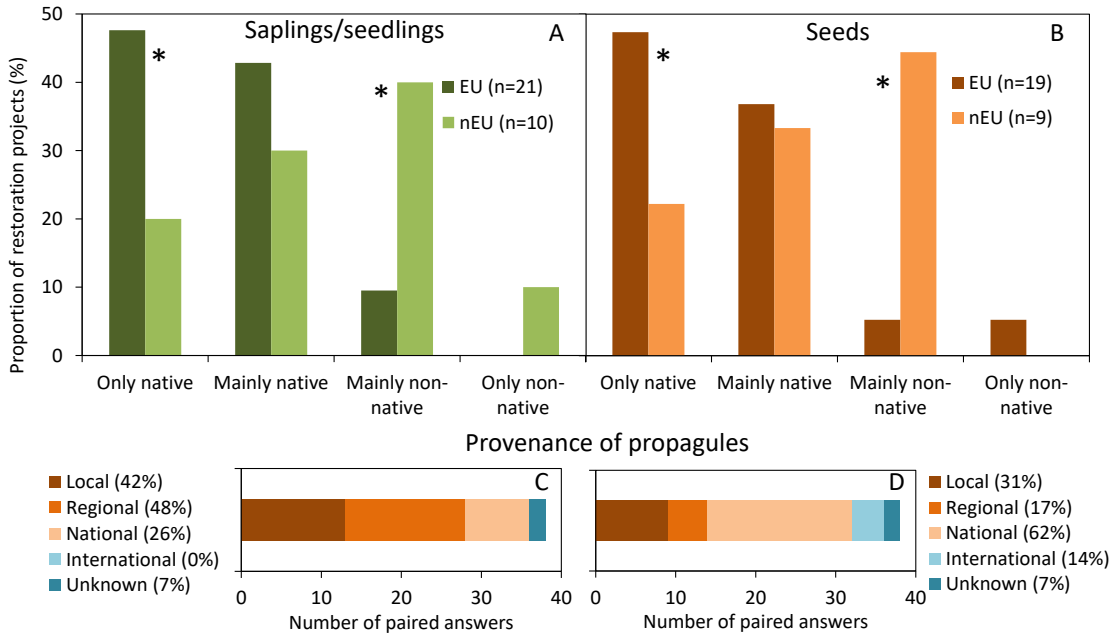

Figure 7.5. *Upper panel:* Proportion of projects using each type of plant species (native vs. non-native) and propagule (nursery-grown saplings/seedlings, A; seeds, B); values are compared between EU and nEU countries (GZLM results, *$p<0.05$). *Lower panel:* Frequency of provenance class reported for saplings/seedlings (C) and seeds (D). Local provenance corresponds to a <10 km distance from the restored site. The relative overall proportion of projects referring to each class of provenances for saplings (C) and seeds (D) is displayed within brackets.

Most projects used nursery-grown saplings of local (42%) and regional (48%) provenance, and only 26% reported the use of saplings of national provenances (Fig. 7.5-C). In contrast, most projects used seeds of national provenance (62%), while only 31% and 17% used seeds of local and regional provenances, respectively (Fig. 7.5-D). The projects using seeds from international sources (14%) were implemented in less arid sites ($p<0.05$) (Table S7.2), while no association was found between the propagules' provenance and degradation causes (Fig. 7.5-D, Table S7.2).

Restoration monitoring and success evaluation

About one third of the projects (31%) did not include maintenance activities after plant introduction, while 27% had activities in the first year, and 37% had extended maintenance up to five years, which was roughly the upper limit reported (Fig. 7.6A). The duration of maintenance activities, including irrigation, control of disturbances (fire, grazing, pests), and control of undesired species through mechanical or chemical methods, showed no relationship with aridity levels (data not shown).

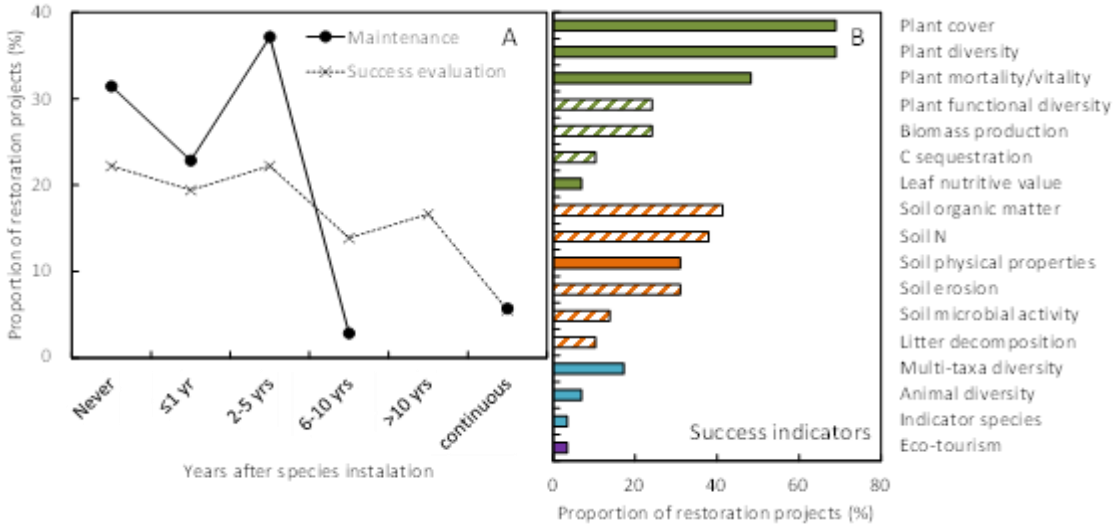

Figure 7.6. Proportion of projects with maintenance and success evaluation activities after plant introduction (A), and indicators used to measure restoration success (B), based on plant (green), soil (orange) and other information (blue and purple); solid bars correspond to non-functional indicators, and striped bars correspond to functional indicators.

Restoration success was never evaluated in 22% of the projects, while about the same proportion evaluated this only in the first year after plant introduction (19%), or until the 5th year (22%). A low proportion evaluated results over the long-term (>6 years, 14%; >10 years, 17%) (Fig. 7.6A). Restoration success evaluation was mainly based on plant cover and diversity (69% of the projects) and plant vitality (48%) (Fig. 7.6B). The main

indicators of ecosystem functioning - hereafter noted as 'functional indicators'- were soil organic matter (41% of the projects) and nitrogen content (38%) (Fig. 7.6B). On average, 4 to 5 different metrics were used per project to evaluate restoration success. The respondent's answers indicated that in 77% of the projects there was no attempt to specifically quantify ecosystem services (data not shown). Restoration efforts yielded partially unexpected results in 50% of the projects (n=18). The main negative unexpected results were high plant mortality associated mostly with drought, low soil quality and erosion, and low or inadequate biodiversity (e.g. dominance of a species), mainly associated with drought (Table 7.3).

Table 7.3. Number of times each probable cause was reported for each unexpected negative result in the restoration projects (number of paired answers) (n=18). The darker the color the higher the value.

Probable causes	High mortality	Low or inadequate biodiversity	Dominance of a native species	Low plant cover	Low natural recruitment
Drought	11	4	3		1
Low soil quality	7	1	1		1
High erosion	3	1	1	1	1
Pests	2	1	1		
Inappropriate planting techniques	2	1	1		
Excessive irrigation		1			
Wildfire	1				
Invasive species		1			
High fragmentation				1	
Short elapsed time				1	

In many cases, restoration projects were considered partially (44%) or completely unsuccessful (6%), i.e. with their aims only partially achieved or not fulfilled at all. Success perception was not related to the background of the respondent nor with the aridity level. The ecological restoration practice index was significantly higher in areas degraded by infrastructure development (e.g. roads) or by industrial activities (quarries) in EU countries than in nEU countries ($p<0.05$) (Fig. 7.7). This was mostly due to a higher use of non-native species and of propagules of exogenous provenances, and to a shorter-term evaluation of restoration success in nEU countries (Fig. 7.7). Restoration after industrial activities had a higher index than that of burned lands in EU countries ($p<0.05$), mainly due to the lack of success evaluation, especially in the long-term (>5 years), and of scientific assistance to restoration, in the latter case (Fig. 7.7). This index was not significantly correlated with the aridity level.

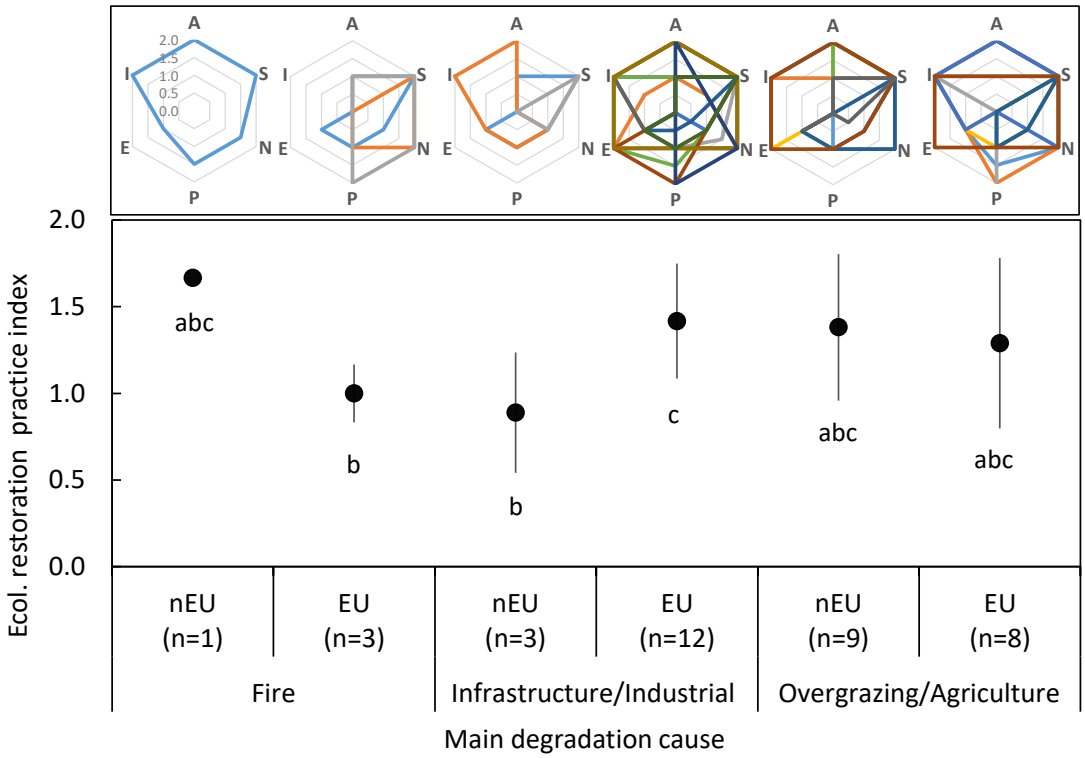

Figure 7.7. *Ecological restoration practice index* (mean ± SE) calculated for each main degradation cause in EU and nEU countries. This index varies from 0 to 2 and is the average of the scores of the answers to six questions related to: scientific assistance (A), soil source (S), species nativeness (N), species provenance (P), timing of success evaluation (E) and success indicator(s) used (I) (see Material and Methods for further details). Different lowercase letters indicate significant differences among means (GLM results, $p<0.05$). The number of projects represented in each group is indicated (n). The *upper panel* shows the score of each question (uppercase letters) for the projects included in each group, and each project is represented by a different color.

7.5 Discussion

This survey across the Mediterranean Basin provided an overview of the current practice of ecological restoration projects implemented in terrestrial ecosystems in the region. General overviews over large geographical areas can provide a critical perception on what is needed to improve restoration efforts. Although the survey was directed towards ecological restoration projects, in some cases it may be difficult to confirm that the type of restoration implemented was 'ecological', in the sense advocated by the Society for Ecological Restoration (SER, 2004). We decided to rely on the practitioner's judgment and we acknowledge this limitation. Nevertheless, we found considerable differences among projects in the degree to which they follow ecological restoration principles.

Many restoration projects implemented in EU countries were primarily motivated by legislation requirements. This is probably related to legal initiatives and policy targets developed in recent years regarding biodiversity conservation within the European Union. In non EU countries (nEU), public opinion and health were more important motivations for ecological restoration projects than in EU countries. Land degradation associated with intensive land use (e.g. overgrazing, intensive agriculture) decreases productivity and has direct negative impacts on people's livelihood and income, which may explain a growing social involvement in restoration issues calling for more sustainable land management approaches (Derak et al., 2016).

The first step of restoration activities concerns soil, as the primary support of terrestrial ecosystems (Costantini et al., 2016). Only 8% of the projects reported the use of non-local soil, and were associated with restoration after infrastructure development (e.g. roads) and industrial activities (e.g. quarries), which often entail the complete removal of topsoil in large areas. In such cases, it is very important to save and properly 'store' original soils to be reintroduced later, in the restoration process, otherwise the use of exogenous soil, although unadvisable, becomes frequently inevitable. The use of local topsoil presents many advantages in comparison with exogenous soil. No matter how degraded, unstructured and depleted it may be, it carries propagules of locally adapted species and the associated soil fauna and flora, exhibiting a higher potential to enhance natural colonization and succession as well as biotic interactions in the restored ecosystem, after active restoration interventions take place. On the contrary, exogenous soils may carry propagules of exotic species, which might lead to unexpected and often negative restoration outcomes (Rowe, 2010; Tischew et al., 2011).

Most of the Mediterranean Basin is subject to low water availability, which severely constrains the natural recovery of vegetation. This might explain why the majority of the projects surveyed relied on the introduction of plant species, presumably to (partially) overcome that limitation and thus promote the subsequent restoration of the whole biological community (e.g. animal species). Restoration projects implemented in EU countries relied more on native plant species (both saplings and seeds) than in nEU countries. This may be related with the need to comply with the aforementioned EU legislation regarding biodiversity conservation. Conversely, nEU countries used, in general, more non-native species. The main reasons for this preference were their frequently higher relative growth rates when compared to native species, and external factors such as a higher commercial availability and lower price, as well as, to a lesser extent, their aesthetic value and alleged nurse-effect as facilitators of the establishment

of other species' (Nunes et al., 2014). Regardless of the legitimacy of these arguments (Davis et al., 2011; Rowe, 2010; Tischew et al., 2011), they diverge from ecological restoration principles, which advocate the use of native species to the greatest practicable extent (SER, 2004). This is not only because indigenous species are adapted to local edaphic and climatic conditions and play a positive role in the network of local biotic interactions, necessary for ecosystem sustainability and resilience (Tischew et al., 2011), but also because the use of exotic species often entails ecological risks and may compromise the success of the restoration actions (Alyokhin, 2011; Rowe, 2010; Shackelford et al., 2013). Used for restoration, such species may become dominant (or favour the dominance of undesired species) and outcompete native species (e.g. Nunes et al., 2014), as reported in some of the surveyed projects. Additionally, they may have a negative impact in biotic fluxes and interactions with local flora and fauna (Alyokhin, 2011), thus hindering natural colonization and succession, which should be promoted and capitalized as much as possible.

Young plants used in restoration activities were mostly of local or regional provenance, probably also for logistic reasons, but plant seeds were not, as is often the case in many restoration projects (Kiehl et al., 2010; Oliveira et al., 2012; Tischew et al., 2011). Although the use of generalist and easily commercially available 'seed recipes' of exogenous or unknown provenances is a common practice particularly in large-scale restoration projects, it may lead to the loss of local diversity of adapted varieties and possibly alter ecosystem functions (Bischoff et al., 2010; Rowe, 2010; Vander Mijnsbrugge et al., 2010). Local plant varieties exhibit morphological and functional traits which determine their fitness (Bischoff et al., 2010), enabling adaptation to the harsh climate of drylands and to other disturbances. Moreover, species traits greatly influence ecosystem functions (Mason and de Bello, 2013). It is therefore advisable to promote genetically diverse local provenances in restoration projects, particularly in the context of harsh environmental conditions (Vander Mijnsbrugge et al., 2010). For such purposes, the availability and ability to collect local seeds should be promoted (Kiehl et al., 2010; Tischew et al., 2011; Vander Mijnsbrugge et al., 2010). In addition, when the quantity of locally collected seeds is insufficient, e.g. for the restoration of large areas, seed collection from habitats with similar climates and geomorphologies, even if distant, might be an option (Vander Mijnsbrugge et al., 2010). The provenance of propagules for restoration projects is expected to be particularly relevant in species adaptation to climate change, which is predicted to severely impact Mediterranean Basin ecosystems (IPCC, 2007). Recent works suggest that, in a climate change scenario, provenances

from slightly different climates might be necessary to facilitate plant adaption (e.g. assisted gene flow) (Breed et al., 2013). However, regardless of its value, this view still lacks consistent evidence from scientific research, e.g. from long-term experiments, to fully assess the feasibility and success of such a seed-sourcing strategy (Breed et al., 2013; Hodgins and Moore, 2016).

Fifty percent of the restoration projects faced unexpected results, such as high plant mortality or low or inadequate biodiversity. This highlights our still low predictive ability concerning restored ecosystems trajectories and outcomes (Suding et al., 2015). Despite recent progress in bringing the science and the practice of restoration closer to each other (Cabin et al., 2010), we need to improve our understanding of ecosystems complexity, and invest further in its integration into ecological restoration practice. In this context, monitoring is an essential tool, as it is necessary to evaluate restored systems trajectories and adopt flexible management strategies whenever necessary (adaptive management) to redirect the restoration course and meet the predefined restoration goals. It is the only way to learn from examples (both successes and failures) and improve restoration practices. Nevertheless, 22% of the projects made no evaluation of the restoration outcome, and only a low proportion (31%) evaluated it for more than six years after plant introduction, coinciding with the end of maintenance activities in the majority of the projects. This makes it impossible to monitor the so-called 'slow variables' (Carpenter and Turner, 2000), some of which are critical to the dynamics of dryland ecosystems and key to their functional recovery (e.g. soil fertility) (Reynolds et al., 2007b). The long-term monitoring of restoration projects depends on the stakeholders involved and particularly on the funding available to implement it. Hence, it is crucial that restoration plans include and ensure funding to support monitoring programs during an adequate number of years after restoration.

Moreover, the success of restoration in most projects was primarily based on plant species cover and diversity (69%), while few projects used functional indicators, despite the relatively frequent measurement of soil organic matter and nitrogen content (41 and 38%, respectively). Functional indicators are directly linked to ecosystem functions, i.e., dynamic attributes affecting the fluxes of energy and mass (solids, water and nutrients), which are the basis for the self-maintenance of an ecosystem. Examples are primary productivity, trophic interactions, decomposition, and nutrient cycling. According to ecological restoration principles, ecosystem functioning is key because it ensures sustainability over the long term. Moreover, the ability to deliver diversified ecosystem services, not only the provisioning services (e.g. food, fuelwood, fresh water),

but also the regulation and maintenance services (e.g. drought and flood buffering, genetic diversity, carbon sequestration), and cultural services (e.g. tourism, recreation) (Haines-Young and Potschin, 2013), depends on ecosystem functioning (Groot et al., 2013; Lavorel and Grigulis, 2012). Hence, functional indicators such as the presence and abundance of critical functional groups or traits, decomposition rates, soil microbiological activity, just to mention some examples, should be included in restoration monitoring programs. Interestingly, although ecosystem services' delivery was frequently pointed out as one of the restoration goals (data not shown), in 77% of the projects no attempt was made to specifically quantify them. Nevertheless, ecosystem services assessment can be a way for practitioners to emphasize socio-economic benefits of restoration as a worthwhile investment for society (Groot et al., 2013). Long term monitoring and evaluation of both biophysical and socio-economic 'slow' variables (Carpenter and Turner, 2000) are therefore important to fully assess restoration success.

The ecological restoration practice index we built, based on six important components of ecological restoration practice, enabled us to conclude that, although all projects claimed to follow 'ecological restoration' guidelines, they varied considerably and differed in some procedures. The areas degraded by infrastructure building or industrial activities had a higher mean restoration practice index in EU countries than in nEU countries, mainly because EU countries, in general, relied more on native species and propagules of local provenance, and on longer evaluation of restoration success (> 5 years), despite the much smaller number of replicates in the second case. This can be associated with EU environmental policies, translated into more legislation targeted at local biodiversity conservation. Restoration after industrial activities also had a higher index than that of lands affected by fire in EU countries, and this was mainly associated with the lack of long term success evaluations and of scientific assistance to restoration in the latter. This may be associated with the high resilience and generally fast recovery of Mediterranean ecosystems after fire, probably weakening the perception of the need for long-term evaluation, despite the small number of projects analysed in these conditions.

The unbalanced geographical distribution of the answers obtained across the Mediterranean region (e.g. a higher number of cases from the Iberian Peninsula) and the low number of replicates in some cases, may have prevented the emergence of clearer or more robust trends regarding restoration practice. Although it is the most used language for scientific communication, English (also used in the questionnaires) may have discouraged the participation of some restoration practitioners (e.g. from

francophone countries in North Africa), particularly those not involved in scientific research.

Conclusions and implications

By collecting information on the practice of ecological restoration projects implemented in terrestrial ecosystems across the Mediterranean region, we identified considerable variability in restoration procedures, in some cases closer to fulfill ecological restoration principles than others. Our work is a step forward in understanding what is going on in restoration practice, and the work has produced some indications on what is needed to improve and promote ecological restoration efforts in Mediterranean areas, particularly in drylands. Sharing technical information about restoration practice, including unexpected results in restoration, problems, and successful solutions, and making it readily available to other practitioners, is crucial to improve restoration practice.

Considering the potential risks of the use of non-native species and of genetically uniform varieties in ecological restoration actions, a cautious approach is required. The use of native species and of local propagules in restoration plans should be promoted, particularly in countries outside the European Union. This calls for increased awareness among restoration practitioners (e.g. technicians, local people) on the importance of such species for local adaptation to climate and other disturbances, particularly in a context of a changing environment, as well as to promote biotic interactions and ecosystem sustainability and resilience. To achieve this, regulated collection and commercial availability of local propagules of native species should be promoted, thus hopefully contributing to reduce their prices.

Monitoring and evaluation should be priorities for all restoration projects, as this is the only means to learn from experience, detect undesirable outcomes and flexibly adopt management strategies to cope with them. Since much is still unclear regarding restored ecosystems' trajectories and evolution, evaluation in the medium/long term is also essential, in order to monitor 'slow' ecosystem variables (e.g. soil fertility) which are often crucial in dryland ecosystems. Hence, it is important to consider and ensure appropriate funding for long-term monitoring of restoration projects. As long as ecological restoration is the aim, an evaluation of the restored ecosystems focused on ecosystem functioning (i.e. using 'functional' indicators) is indispensable to assess and ensure, as far as possible, their sustainability and resilience over the long term, particularly under a climate change scenario. Considering that ecosystem services' delivery was frequently pointed out as one of the restoration goals, the inclusion of indicators of ecosystem

services in monitoring protocols would match this claimed goal, as well as increase society's awareness of the importance of restoration.

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Supporting Information

Table S7.1. Description of the surveyed restoration projects.

Project Name/ Description	Aridity	Country	LAT	LONG	Degradation cause
Sand dune restoration	Arid	Israel	30.56	34.23	Overgrazing, Vehicle circulation
Restoration of abandoned sheep corrals	Arid	Israel	30.98[a]	34.94[a]	Trampling
Demonstration project on strategies to combat desertification in arid lands with involvement of local agropastoral communities	Arid	Tunisia	34.80[a]	9.80[a]	Deforestation, Overgrazing, Climate
Sustainable land management	Arid	Tunisia	33.13[a]	9.70[a]	Infrastructure development, Deforestation, Intensive agriculture, Overgrazing, Climate
Fighting land degradation	Semi-arid	Algeria	27.99	1.64	Infrastructure development
Quarry restoration	Semi-arid	Greece	40.64[a]	22.93[a]	Industrial activities
Rehabilitation of limestone quarries	Semi-arid	Greece	40.46[a]	23.40[a]	Industrial activities
PRACTICE	Semi-arid	Italy	38.99	8.91	Deforestation, Overgrazing, Fire
Participative ecological restoration of forest in Beni Boufrah	Semi-arid	Morocco	35.15	-4.32	Deforestation, Intensive agriculture
Demonstration project on strategies to combat desertification in arid lands with involvement of local agropastoral communities	Semi-arid	Morocco	34.28[a]	-3.67[a]	Deforestation, Overgrazing, Climate
Conservation of biodiversity	Semi-arid	Palestine St.	31.76[a]	35.25[a]	Infrastructure development, Land use
REDMED	Semi-arid	Portugal	37.35	-7.45	Deforestation, Intensive agriculture, Fire
Quarry restoration: Cerro da Cabeça Alta, Milhanes and Passagem	Semi-arid	Portugal	37.14	-8.10	Industrial activities, Infrastructure development, Deforestation

Project Name/ Description	Aridity	Country	LAT	LONG	Degradation cause
Experimental soil restoration and erosion control in limestone quarries under arid climates	Semi-arid	Spain	36.92	-2.50	Industrial activities
RECUVES	Semi-arid	Spain	38.17	-0.86	Intensive agriculture, Fire
AMID	Semi-arid	Spain	39.21	-6.33	Overgrazing
Ecological restoration of a limestone quarry	Semi-arid	Spain	39.92	-3.65	Industrial activities, Intensive agriculture
Cover crops in olive orchards and vineyards	Semi-arid	Spain	40.07	-3.52	Intensive agriculture, Land use
REACTION	Semi-arid	Spain	38.23	-0.90	Infrastructure development , Intensive agriculture, Land abandonment
Afforestation to protect soil from erosion	Semi-arid	Spain	37.90	-1.50	Deforestation, Intensive agriculture, Overgrazing, Fire, Climate
Soil conservation (fight against erosion)	Semi-arid	Spain	37.94	-1.54	Intensive agricultutre
RECARE	Semi-arid	Spain	39.48	-0.37	Fire
Albatera restoration pilot project	Semi-arid	Spain	38.23	-0.92	Deforestation, Overgrazing
CASCADE[b]	Semi-arid	Cyprus	34.74	32.65	Deforestation, Fire
CASCADE[b]	Semi-arid	Greece	35.04	24.92	Deforestation, Fire
CASCADE[b]	Semi-arid	Italy	40.64	16.19	Deforestation, Fire
CASCADE[b]	Semi-arid	Spain	38.76	-0.86	Deforestation, Fire
Restoration of dry steppe pastures	Dry subhumid	Armenia	40.18	44.58	Overgrazing, Climate
Study and mitigation of soil disturbance and land degradation caused by fires	Dry subhumid	Bulgary	41.88	25.31	Fire
Developing agropastoral systems in degraded rangelands	Dry subhumid	Lebanon	34.11	36.39	Overgrazing

Project Name/ Description	Aridity	Country	LAT	LONG	Degradation cause
Herdade da Mesquita sand and clay pits restoration	Dry subhumid	Portugal	38.52	-9.09	Industrial activities
Quarry restoration at Secil	Dry subhumid	Portugal	38.49	-8.94	Industrial activities
New technologies of raising forest plantations and orchards in harsh environmental conditions of Ramsko Golubačka peščara Sands	Dry subhumid	Serbia	44.72	21.56	Fire, Climate, Moving sands
Resistance to reclamation of wildland	Dry subhumid	Turkey	37.58[a]	36.93[a]	Infrastructure development, Overgrazing
Using of bioenergy crops, soil erosion and desertification	Not dryland	Georgia	42.27[a]	43.35[a]	Deforestation, Intensive agriculture, Overgrazing
Monitoring soil bioengineering projects in Vesuvius national park	Not dryland	Italy	40.81	14.43	Infrastructure development, Erosion
CASCADE[b]	Not dryland	Portugal	40.59	-8.17	Deforestation, Fire
Slope restoration at A21 Malveira junction	Not dryland	Portugal	38.94	-9.24	Infrastructure development, Erosion
Salamonde II: dam margin erosion prevention	Not dryland	Portugal	41.68	-8.09	Infrastructure development
Restoring degraded areas at Serras de Aire e Candeeiros natural park	Not dryland	Portugal	39.51	-8.79	Industrial activities

[a] Coordinates refer to a general location (regional or country level)

[b] Restoration sites belonging to one single project (one questionnaire)

BSC: Biological Soil Crusts

Table S7.2. Results of the generalized linear models using binomial distribution with logit link function (estimate ± SE, and p-value significance) to test the importance of explanatory variables for the categories of response variables, codded as dummy (0 and 1). Explanatory variables: *Aridity* corresponds to the value of the aridity index; *Location* is a binary variable differentiating European Union (EU) from non-EU countries; *Degradation cause* has 3 categories (Overgrazing/agriculture, Infrastructure/Industry and Fire). In 8 cases for which the provided project location coordinates were too general to accurately estimate the aridity index, we used the median of the aridity index for the corresponding aridity class: arid = 0.125, semi-arid = 0.35, dry subhumid = 0.575. * p<0.05; **p<0.01; ns: not significant.

		Explanatory			
Response	**Dummy variable/ contrast**	Aridity	Location	Degradation cause	Location x Degradation cause
Degradation cause	Overgrazing	ns	ns	–	–
	Agriculture	ns	ns		
	Infrastructure/ Industrial	ns	ns		
	Fire	ns	ns		
Restoration motivation	Governmental initiative	–	ns	–	–
	Legislation requirements		2.33±0.88**		
	Public opinion/ health		-2.71±0.86**		
	Enterprise initiative		ns		
Soil source	Non-local	ns	ns	ns	ns
	Non-local + spoil materials	ns	ns	ns	ns
Revegetation techniques	Hydroseeding	ns	ns	ns	ns
	Biological soil crusts			ns	ns
Species type - seedlings	Only native	ns	2.30±1.16*	ns	ns
	Mainly native	ns	ns	ns	ns
	Mainly non-native	ns	-2.30±1.16*	ns	ns
	Only non-native	ns	ns	ns	ns
Species type - seeds	Only native	ns	2.89±1.36*	ns	ns
	Mainly native	ns	ns	ns	ns
	Mainly non-native	ns	-2.89±1.36*	ns	ns
	Only non-native	ns	ns	ns	ns
Propagules' provenance	National or International	ns		ns	ns
	International	3.94±2.01*		ns	ns

Table S7.3. Number of paired answers and relative proportion of who planned and performed the restoration activities. The darker the color the higher the value.

	Implemented								
Planned	Employees involved enterprise	Private restoration enterprise	Local municipality	Regional government	Conrserv./Forestry state institute	Scientists/researchers	NGOs	Citizens/Local farmers	Tot. percentage
Employees of the involved company	8	2	1		1		1		12.3%
Private restoration companies	3	3					1	1	7.5%
Local municipality			4				1	2	6.6%
Regional government				1	1			2	3.8%
Conservation/Forestry state institute	3		1	1	8	1	1	2	16.0%
Scientists/researchers	10	5	5		5	5	5	6	**38.7%**
NGOs	2	1	1				3	2	8.5%
Citizens/Local farmers	1	1	2					3	6.6%
Total percentage	**25.5%**	11.3%	13.2%	1.9%	14.2%	5.7%	11.3%	17.0%	

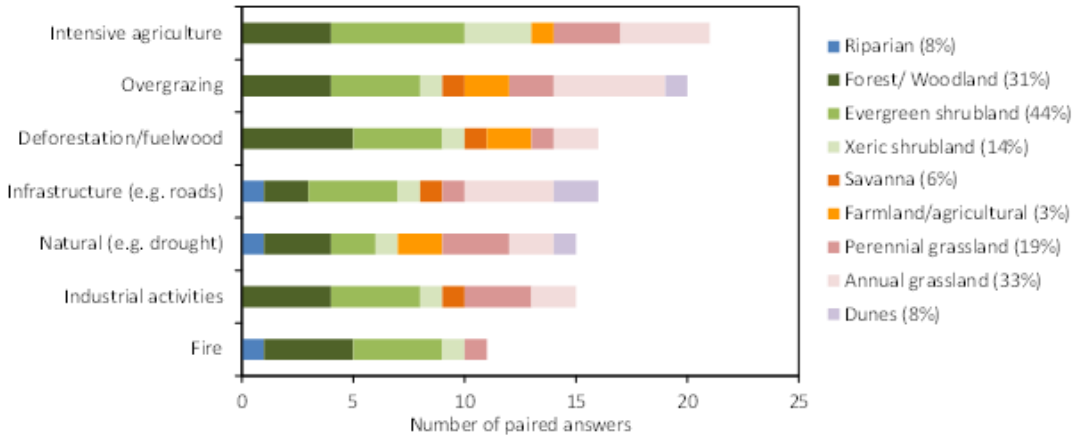

Figure S7.1. Number of times each habitat type was reported in association with the main degradation causes assessed by the survey (number of paired answers). The overall relative proportion of restoration projects referring to each habitat type is displayed within brackets.

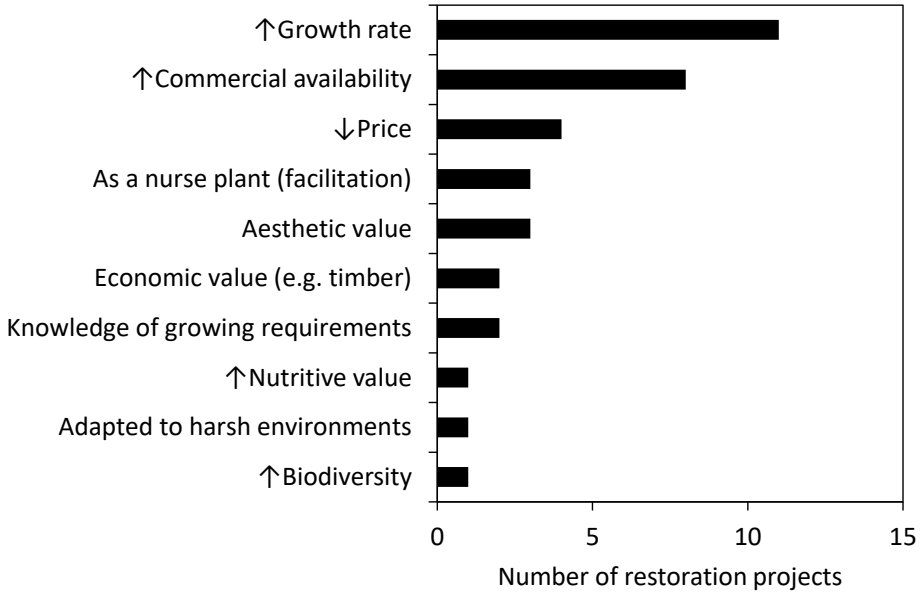

Figure S7.2. Reasons for using non-native species in the surveyed restoration projects.

Chapter 8

General Discussion

8 General Discussion

Climate change models forecast an overall increase in aridity in drylands, in the near future. Drier conditions are expected to increase the vulnerability of drylands to desertification and land degradation, hampering ecosystems' functioning, and the delivery of ecosystem services. Hence, it is crucial to understand and predict the consequences of climate change for dryland ecosystems. Functional traits determine species' responses to environment, and their influence on ecosystem processes, thus providing a more mechanistic and universal way to track the impact of climatic variation on ecosystems, than species diversity alone.

The main aim of this thesis was to model the response of Mediterranean dryland ecosystems to climate based on plant functional traits, using a spatial climatic gradient, to predict changes over time due to climate change. In addition, it aimed at using this information to select a trait-based indicator to track climate change effects on dryland ecosystems at a global scale, and contribute to the improvement of land management strategies and restoration tools to mitigate desertification and land degradation in drylands.

Our work contributed to advance the knowledge on plant functional response to desertification and land degradation due to aridity, and to understand its variation in space and over time. We developed a functional diversity-based indicator of ecosystem functioning loss due to aridity to be used at a global scale, fulfilling a claimed need of the United Nations Convention to Combat Desertification (UNCCD), and demonstrated the need of a better integration of trait-based ecology into dryland restoration.

To achieve the proposed goals, we went through several research steps sequentially. Firstly, to efficiently quantify functional trait variation in response to climate, we needed a precise and reproducible method. We compared the performance of different methods to sample trait relative abundance in the field, and demonstrated that the point-intercept method was the best to be used for trait quantification at a global scale. Then, we aimed at selecting plant functional traits responding to climatic variation. Using the method selected initially, we studied how several plant functional traits varied along a spatial climatic gradient, adopting a 'space-for-time substitution' approach. We identified the main plant traits responding to aridity, associated to plant strategies to survive, reproduce, disperse and regenerate in drylands. We found that aridity affected the mean of a group of traits in different directions (increase or decrease), and caused a decrease in the functional diversity of all traits, more evidently for some of them.

Afterwards, as we used ‘space-for-time substitution’, we needed to check if space was a good proxy for time, particularly in dryland ecosystems, where inter-annual climatic variability is high. We confirmed that inter-annual climatic fluctuations greatly affect functional traits, particularly their means, suggesting that functional changes are transitory, recovering quickly during wetter years. Nevertheless, trait functional diversity was reduced by the worsening of climatic limiting factors for most traits, and its inter-annual variation was more influenced by past climate at each site, than trait mean. Hence, we hypothesized that if dry conditions prevail in time, changes over time would approach those found along space, i.e., we would find a lower functional diversity under more limiting conditions stable over time. Yet, we only evaluated changes along four years, and would need longer data series to confirm this assumption. Considering that functional diversity showed a consistent decreasing trend with the worsening of climatic limiting factors for most traits, we built a multi-trait functional diversity index, and modeled its relationship with aridity, using data from a high-resolution spatial climatic gradient. We found a monotonic non-linear decrease in functional diversity with increasing aridity. We demonstrated it is a better indicator of aridity impacts on ecosystems than taxonomic diversity, which can be used to map areas at risk of desertification and land degradation, where mitigation and restoration efforts should focus.

However, aridity explained only a part of trait variation, suggesting that other drivers were also influencing them. Knowing that the effect of climate on plant communities is modulated locally by soil and topographic characteristics, we explored the relative effect of these drivers, and found that topo-edaphic factors played a major role shaping trait mean and range, particularly of traits associated to shrub encroachment, i.e. involved in changes between shrub and herbaceous dominance. Since climatic factors had only a minor role in these changes, it allowed us to presume that climate change will not have a strong impact on shrub encroachment in Mediterranean drylands. Finally, we provide a comprehensive overview of the current restoration practice in Mediterranean drylands, showing that trait-based ecology is still poorly used in practice, particularly in restoration monitoring, where it could be a useful indicator of ecosystems functional recovery.

8.1 A universal method to sample plant functional traits in drylands

A common plant trait sampling method that can be used to track the impacts of aridity on different dryland ecosystems will allow to understand the universal and specific aspects of the ecology of drylands, including the development of global trait-based indicators of land degradation due to aridity to be used, for example, at the United Nations Convention to Combat Desertification (UNCCD) level. Our work allowed the selection of the best field method to quantify the abundance of plant traits in dryland communities, which is required to estimate functional trait metrics. The use of a reproducible method is essential to enable the comparison of functional metrics between different plant communities at a global scale to be compared in space and over time, and to be evaluated by different people. The method we selected – the point intercept method – is particularly suitable to sample plant communities with a grassy-type understory mixed or not with shrub patches, such as grasslands, savannas, and woodlands, the most representative vegetation physiognomies found in drylands (Reynolds et al., 2007). This method showed higher precision in the quantification of the relative abundance of traits, in relation to the other methods studied. In addition, it allowed the analysis of a greater number of species within the 80% 'dominance' threshold, considered an adequate proportion to characterize functionally a plant community (Garnier et al., 2004, Pakeman and Quested, 2007). Therefore, it proved to be better to perform fine-scale monitoring of functional traits in dryland plant communities.

Functional traits are a 'universal approach' in the sense that they can be studied in any type of community, independently of the species identity. Dryland ecosystems are spread all over the world in different geographical areas. These areas, regardless of how distinct they may be in terms of species assemblages, share a common problem, the vulnerability to desertification and land degradation due to high aridity levels and human presence, which are expected to increase in the near future. We can find examples of drylands in all continents, from Caatinga which is a mosaic of xeric shrubland and seasonal dry forest in the semi-arid northeast of Brazil (Leal et al., 2005, de Albuquerque et al., 2012), to Miombo tropical woodlands in eastern and southern Africa (Sileshi et al., 2007), to Australian woodlands (Ludwig and Tongway, 1995). To study global problems, we need common methods. With this work we proposed the point intercept method to be used at the global scale.

8.2 Which trait values should be used?

The quantification of functional traits in communities is used to 'weight' trait values (mean or range) or categories by their abundance, to obtain trait-based metrics. Plant trait data may be obtained locally, using standard methodologies (Pérez-Harguindeguy et al., 2013), or retrieved from scientific literature or trait databases (Kattge et al., 2011). The first approach is considered crucial in the study of processes acting at the plot-scale (e.g. niche partitioning), while the use of database values is considered acceptable for studies at the site-level or at broader scales (Cordlandwehr et al., 2013). In this work, we used on-site trait measurements for some traits, and values from databases or scientific literature for others, along a large climatic gradient. Given that measuring species traits is often laborious and time consuming, and not always possible, trait databases are expected to support the change in paradigm from species to trait-based ecology, to be used at a global scale. However, trait data available in databases is still insufficient, and lacks a large geographical coverage (Kattge et al., 2011). Trait data gaps are more pronounced, for instance, in northern and central Africa, parts of South America, southern and western Asia (Kattge et al., 2011). Thus, trait-based studies should contribute to fulfill trait data gaps as much as possible. Our work used traits from databases and also contributed to improve trait data availability for Mediterranean species, particularly regarding plant height measurements.

When studies are focused on the turnover of species (and their traits) in communities and compared across space or through time, the most frequent procedure is to use one mean trait value per species. Likewise, in this work we used a single trait value per species, ignoring trait variation within species. However, some traits are more plastic than others, and may show a high intraspecific variation along environmental gradients (e.g. specific leaf area). Intraspecific trait variation is expected to be more relevant in studies developed at a local to plot-scales (Albert et al., 2011, Cordlandwehr et al., 2013), and less important in studies involving broad interspecific comparisons (Shipley et al., 2016). It is important to consider that the degree and speed of compositional turnover may differ substantially between different dryland plant communities. It may be fairly rapid in some cases (e.g. 2-5 years), while in others it may take much longer (e.g. 40 years without change), depending on their characteristics (e.g. soil properties) and on how climate and disturbance pressures (e.g. fire, grazing) affect successional or regressive trends (Noy-Meir, 1973). We found considerable species turnover along the spatial climatic gradient studied (median of Sørensen dissimilarty index ca. 0.6), indicating high heterogeneity in specific composition between of sites (Fig.

8.1a, b). Therefore, the use of a trait value per species is likely to capture most of the functional trait variations along the climatic gradient. Nevertheless, some species which can be found virtually along the entire gradient may show considerable phenotypic plasticity along climatic gradients, e.g. *Brachypodium distachyon* (L.) P.Beauv. (Aronson et al., 1992). Hence, intraspecific trait variability should be also considered: (i) for more ubiquitous species showing high plasticity along environmental gradients, (ii) in communities with a low turnover in species composition, or (iii) in studies addressing trait variations at finer spatial scales.

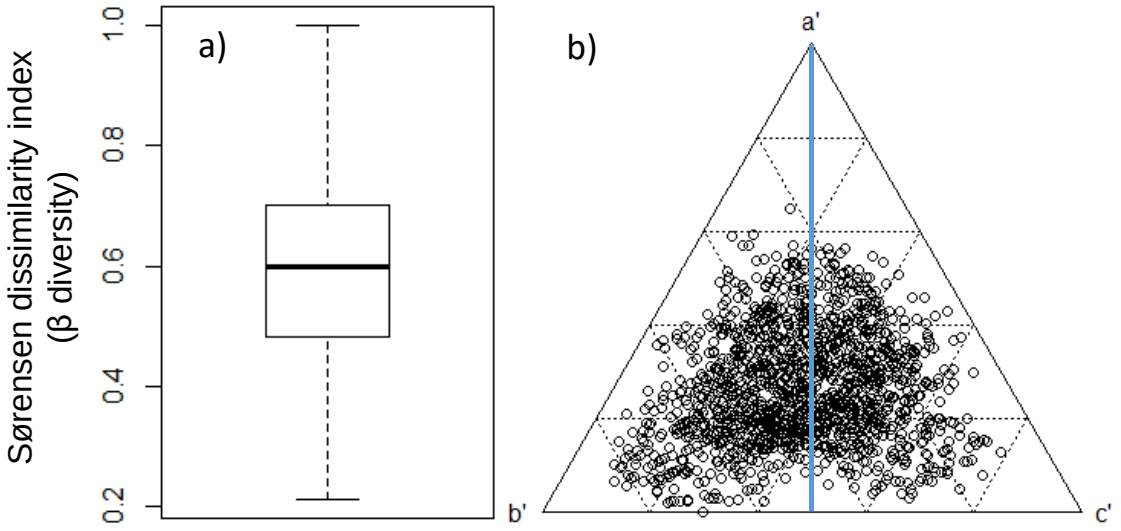

Figure 8.1. a) Variation in Sørensen dissimilarity index along the spatial climatic gradient studied (pairwise dissimilarities between 54 sites) used as a measure of changes in species composition (β diversity). It is calculated as b+c/2a+b+c, where a represents the species in common between a pair of sites, and b and c are the species exclusive of each site of the pair (Oksanen, 2016). **b)** Graphical representation of the contributions of a, b, and c components of β diversity: points which lie close to the vertical blue line represent more similar sites; the further away the points are from the line, to the left or to the right, the greater the dissimilarity between (pairs of) sites (Koleff et al., 2003).

8.3 Which plant traits should be used to track climate in drylands?

The functional traits selected for a particular study should be the ones more responsive to the limiting factors observed along the studied gradients. Changes in the

intensity of the limiting factors should affect the fitness of the plants holding those traits (i.e., affect the probabilities of dispersal, growth, survival, and reproduction), and thus their relative abundances in the community. In our work, we addressed a large number of traits thought to be related to the main limiting factors for plant communities in drylands, which are high and often unpredictable fluctuations in water availability, along with seasonally limiting temperatures (high or low) to their survival, growth and regeneration. These factors may lead to the dominance of particular traits which give species a competitive advantage in resource-utilization under such circumstances, or allow the coexistence of species with specialized niches displaying different traits. Under drier conditions, plant species may exhibit stress-tolerant or stress-avoidant strategies, which involve differences e.g. in their life cycles, height, root depths and specific leaf area (Ackerly et al., 2002, Gross et al., 2013, Costa-Saura et al., 2016). Drier conditions are likely to affect also the proportion of plants with different growth-forms (Fay et al., 2002), the timing and duration of flowering events (Aronson et al., 1992, Kigel et al., 2011, Hänel and Tielbörger, 2015), and plant regenerative strategies involving dispersal and seed traits (Baker, 1972, Arroyo et al., 2006, Volis and Bohrer, 2013, Gremer and Venable, 2014). We addressed plant traits involved in all these strategies, i.e. relevant in dry environments.

We found important shifts in trait community means (CWM) in response to aridity, namely an increasing proportion of annual species, and of plants with lower maximum height and shorter flowering duration. These traits may be seen as reflecting stress-avoidant strategies, i.e. the ability to avoid unfavorable periods, e.g. persisting in the form of seed, ‘scheduling’ their growth and reproduction to periods during which the availability of resources is most likely. We also found an increase in anemochorous dispersal at drier sites. The ability to disperse at longer distances is advantageous to cope with high spatial heterogeneity in resource availability, e.g. due to varying distribution of precipitation in space and over time. However, different co-optimal combinations between dispersal strategies and seed traits may be selected to cope with climatic unpredictability (Volis and Bohrer, 2013). For instance, heavier seeds with high persistence and dispersed by gravity (barochory), able to endure viable in the soil for several years, can be another strategy to deal with climatic fluctuations (Volis and Bohrer, 2013). Hence, despite the dominating trend we found towards anemochory under higher aridity in this work, we can not discard the hypothesis that other strategies, involving different combination of these traits, stand out in other dryland ecosystems. Drier conditions led to a higher proportion of specie with rosette growth-form, suggesting

that a prostrate habit may confer higher ability to withstand dry periods. This trend is likely to be common in dryland ecosystems in general.

In the ecosystem studied, annual grasslands dominating the understory play a decisive role in ecosystem functioning, namely in biomass production and nutrient cycling. Annuals are an important component of drylands (Noy-Meir, 1973, Aronson et al., 1992), and increase in abundance from mesic to more arid conditions, e.g. in western African savannahs (Bates, 2014), or in North American grasslands (Cleland et al., 2013). However, dryland ecosystems may be also dominated by perennial grasses, e.g. semi-arid grasslands of southern Chihuahuan Desert (Reichmann et al., 2013), Patagonian steppes (Yahdjian and Sala, 2002), or Ibero-North African steppes dominated by *Stipa tenassissima* L. (Cortina et al., 2009). In perennial grasses, vegetative regeneration (through meristematic tissue) is thought to be the main regeneration strategy, while regeneration through seeds is less effective (Klimes, 2007). So, clonal and bud bank traits could be important there (Klimešová et al., 2011).

In Caatinga semi-arid ecosystem in northeast of Brazil, for instance, vegetation physiognomies range from predominantly herbaceous to arboreal (de Albuquerque et al., 2012). However, after prolonged droughts, which may endure a few years with very scarce precipitation (Leal et al., 2005, de Albuquerque et al., 2012), the herbaceous component becomes very inconspicuous (virtually absent) (Leal et al., 2005). It only recovers when it rains, and may be quite ephemeral. In addition, most woody species are deciduous, shedding practically all their leaves during these dry periods (Leal et al., 2005). On one hand, this might hamper the study of some traits, e.g. leaf traits, during such periods. On the other hand, it may indicate, for instance, that in such ecosystems most herbaceous species have a long seed persistence, and that leaf longevity may be an important trait to consider. In Brazilian Caatinga, as well as in other dryland ecosystems mostly in America and Africa, we may find a considerable proportion of succulent species (e.g. Cactaceae), which is not the case of Mediterranean Basin drylands. Succulents share with annuals shallow roots, but with higher lateral root spread (Schenk and Jackson, 2002), absorbing water from the uppermost layers of the soil. The occurrence of precipitation during warmer periods, or on finer textured soils, lead to shallower water profiles, which may favor shallow-rooted species (Sala et al., 1997), such as annuals or succulents. In fact, despite aridity effects, we found precipitation during summer to have a significant role on functional trait metrics for some traits. Hence, precipitation seasonality is an important climatic driver to consider when comparing different dryland ecosystems.

Trait functional diversity (FD) showed a consistent decreasing trend with increasing aridity for all the traits we studied. We found a stronger reduction for life-cycle, specific leaf area, onset of flowering, dispersal strategies and seed persistence. This points out to a filtering effect of aridity, reducing the abundance, or filtering out of the community, species with less 'successful' strategies to cope with severe drought. According to our results, the reduction found in FD as a result of environmental filtering, is likely to be common to all dryland systems, even if others traits, different from the ones we studied, are considered.

8.4 Single-trait or multi-trait indicator?

Several indices have been developed over the last decade, aimed at resuming the diversity of multiple functional traits within a community, in a single functional diversity value (Villeger et al., 2008, Laliberté and Legendre, 2010). These efforts aimed at allowing to assess the 'relative position' of species in a multidimensional functional trait space, as well as to estimate the 'functional trait space' occupied by a community, thus facilitating the comparison of functional diversity between different communities, e.g. along environmental gradients. However, despite the merits of this intention, it presents some pitfalls. Different traits may show divergent responses to environmental variation, and condensing them in one index would obscure single-trait trends, or hinder the ecological interpretation of their variation (Butterfield and Suding, 2013). This may be due, for instance, to trade-offs among species strategies. However, in our case, all the traits studied showed a similar negative trend in FD with increasing aridity. Therefore, it makes sense to summarize the information of multiple traits in a joint index. Another point to consider is that different traits may be redundant, i.e. convey the same information. In this case, by joining them in a single FD value, we may be overestimating their importance in relation to other traits. Two solutions may be adopted to avoid redundancy. The first one is to condense the information of single-traits FD into main axis of functional specialization, through techniques that reduce trait data dimensionality in the most informative variation axis, e.g. using multivariate methods such as principal components analysis, as we did in chapter 6. Alternatively, and considering that correlated traits may not give exactly the same information, one may include all traits in a composite index, giving a lower weight to correlated traits, as we did in chapter 3. However, in either case, it is important to start by checking for each particular context whether the considered traits co-vary or not, as species may show different combinations

of traits under different environments, to maximize their performance (Maire et al., 2013, Volis and Bohrer, 2013).

8.5 Does the trait-based indicator respond in a predictable way to climate?

We found a consistent relationship between plant functional diversity and aridity in space, in contrast with species diversity, which showed an idiosyncratic trend. Functional diversity (FD) is expected to positively influence ecosystem functioning. Hence, a decreasing trend in FD is consistent with the loss in ecosystem functioning under higher aridity levels, confirming functional diversity as a good indicator of desertification and land degradation due to aridity. Functional diversity revealed a monotonic non-linear decrease with increasing aridity. Beyond a critical threshold – when the aridity index goes approximately below 0.51 – this decrease in functional diversity is more pronounced. The indicator enabled to map areas according to the functional diversity of plant communities, and identify areas at risk, were functional diversity is low, indicating reduced ecosystem functioning and provision of associated ecosystem services to local population (Fig. 8.2a, b).

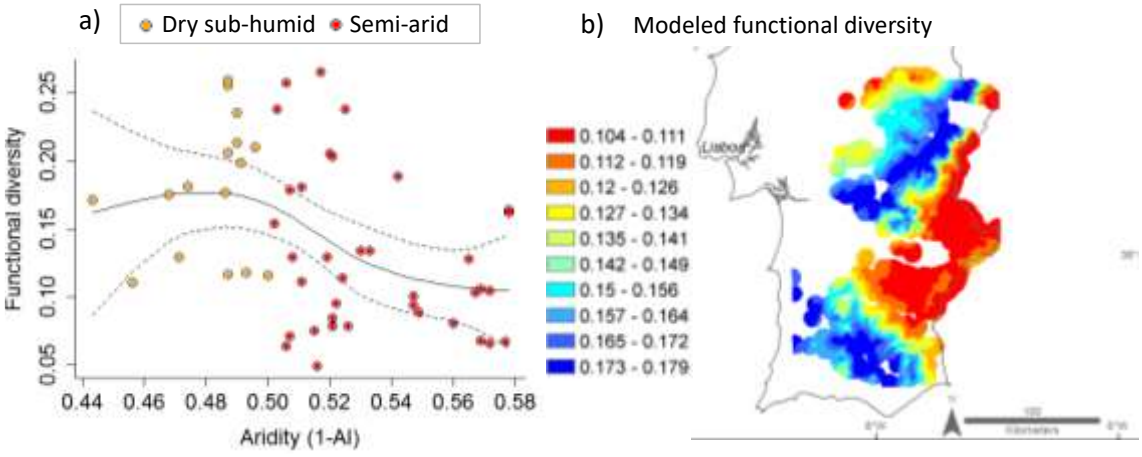

Figure 8.2. a) Variation in multi-trait functional diversity in response to aridity (1 – aridity index), calculated for the traits more responsive to aridity (i.e. 5 traits, with the attribution of lower weights to correlated traits). The graph shows generalized additive model fits (solid lines) and 95% confidence intervals (hatched lines); dimension of the fitted spline (k=4), effective degrees of freedom (edf=2.53), percentage variance explained (21.3%), and significance-level (p<0.01). **b)** Mapping of plant community functional diversity based on the model described in (a), under current aridity values.

8.6 Can the indicator be applied globally?

Regardless of the effect of aridity on plant communities' functional traits, we found them to be influenced by local environmental factors, namely by topographic and edaphic characteristics. This may limit the application of the trait-based indicator in other dryland regions, as these factors will most certainly vary. One solution to deal with this, is to invest in the homogenization of the unwanted confounding factors at the field sampling design phase, e.g. select as much as possible sites with similar topographic and edaphic conditions, in an attempt to isolate the factor of interest, i.e. aridity. When this homogenization of confounding environmental factors is not possible, or desirable, the alternative is to integrate the possible additive or interacting effects of other factors in modeling. However, while topographic characteristics are quite easily mapped for any region and at larger spatial scales using remote sensing data (e.g. digital elevation models), soil characteristics are more difficult to assess for large areas. It may not be feasible to perform a timely and comprehensive analysis of soil properties for large areas, especially considering spatial soil heterogeneity in most drylands. Hence, we need a measure which integrates information on topography, soil properties, and climatic variations, to determine soil water availability patterns, at different soil depths and with high temporal resolution. Recent attempts have been made to fulfill this need, e.g. with the development and continuous improvement of topographic wetness indices (Sørensen et al., 2005), soil aridity indices (Costantini et al., 2009), or simulation models of ecosystem water balance, which also consider biotic processes (e.g. plant interception and transpiration) (Parton, 1978, Schlaepfer et al., 2012). Although all of them present some challenges or limitations to their successful application (Hickler et al., 2009), they undoubtedly constitute significant progress towards achieving this objective. The use of such proxies of soil water availability patterns with high spatial and temporal resolution would enable the application of the trait-based indicator globally, and improve its predictive ability of climate change impacts on desertification and land degradation in drylands.

8.7 Is space a good proxy of time?

Space-for-time substitution relies on the assumption that ecosystems will change in response to climate through time the same way they vary across space presently (Blois et al., 2013). If so, spatial relationships between climate and biodiversity can be

used to project temporal trajectories of biodiversity under changing climate. In this work, we studied variations in plant functional traits along four climatically contrasting years, to test our trait-based indicator developed along space. We found a considerable variability in trait metrics due to climatic fluctuations between years, mainly for mean trait values (CWM). Variations in trait means following the intensification of the most limiting climatic factors over time, i.e. drier conditions or lower winter temperatures, did not show the same response patterns found for the effect of aridity along space for all traits. This means that, while some traits showed the same direction of shift under more limiting climatic conditions, due to short-term and/or long-term (aridity) climatic variation, others displayed contrasting trends. Hence, we found that variations over time in plant trait means (on the short-term) do not match those observed along space.

Functional diversity also varied over time with inter-annual climatic fluctuations but, for most traits, the pattern of change was consistent with the spatial response to aridity, i.e. more climatically limiting conditions led to lower functional diversity. However, these changes were transitory over time, meaning that functional diversity increased during wetter years. These results suggest a high resilience of Mediterranean dryland ecosystems to short-term climatic fluctuations. Previous studies pointed in the same direction, reporting a high resilience of productivity and species composition of the herbaceous understory of Mediterranean Oak woodlands to short-term drought, induced by the field manipulation of precipitation distribution along the year (Jongen et al., 2013a). The authors explained this resilience with the coexistence of different physiological and morphological strategies to cope with drought (Jongen et al., 2015). However, vegetation was not able to buffer long drought periods, which reduced their productivity and affected species composition (Jongen et al., 2013b). We found the response of functional diversity to short-term climatic fluctuation to be influenced by past climate. This indicates a legacy effect from long-term climate at each site, already hypothesized for drylands (Schwinning et al., 2004), and reported in recent studies for primary production in dryland ecosystems (Sala et al., 2012). The authors suggest that the temporal models describe the transient response of primary production to climate change and that the spatial model describes the equilibrium condition to which temporal trends converge, when ‘slower ecosystem variables’ such as the relative species abundance start changing (Sala et al., 2012). This hypothesis also fits our results on functional diversity. Despite transitory changes on the short-term, we expect temporal trends to resemble predictions of the spatial model if climatic conditions persist. We analysed changes over time in a five year period, and this hypothesis could only be

confirmed with long-term data sets obtained under natural climatic conditions or added by manipulation of rainfall amounts in the field (Dunne et al., 2004). Nevertheless, our results support to a moderate extent this assumption. Variations in functional diversity of onset of flowering, for instance, show that temporal trends resemble spatial patterns. Wetter sites showed lower variability between years and consistently higher functional diversity, while drier sites, despite a much higher variability between years, showed the tendency for a lower functional diversity in dry years in comparison with 'average' or wetter years (Fig. 8.3). How can we obviate this lag between variation through time and across space, to allow the use of a trait-based indicator? We suggest as a possible solution to sample plant communities during a climatically average-to-wet-year, and analyze the results taking into consideration the past climate at that site. This would allow to compare the current functional diversity between sites subject to different aridity levels over the past. If drier sites over the past exhibit significantly lower functional diversity than historically more humid ones, during a climatically 'good year', this may indicate a loss in ecosystem resilience. This lag between temporal and spatial variation in functional diversity limits the use of a trait-based indicator as an early-warning system, as functional alterations would have to actually occur, to be detectable. Nevertheless, they constitute a good indicator to map areas at risk of severe loss in functional diversity, and thus at risk of desertification and land degradation.

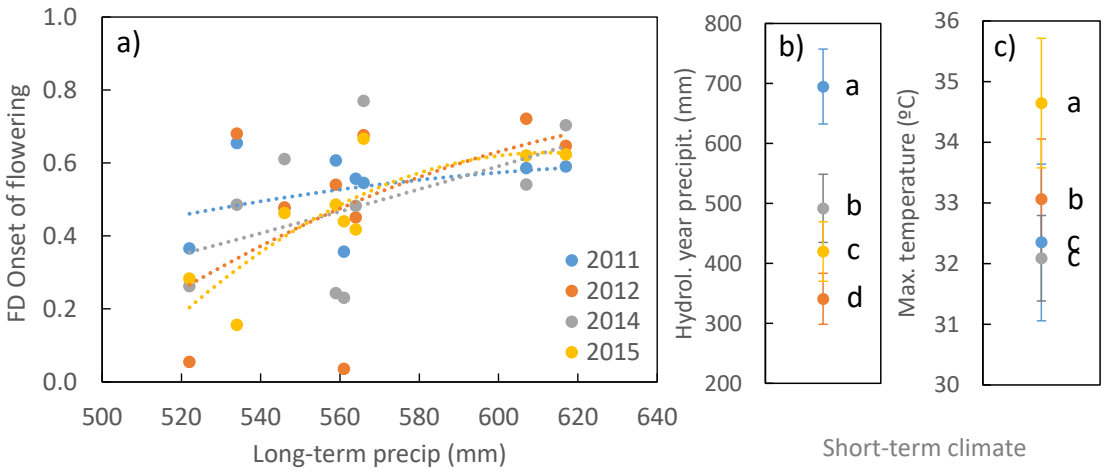

Figure 8.3. a) Variation of functional diversity of onset of flowering in relation to long-term precipitation (average of 50 years), for the four years of the study; **b)** mean annual precipitation and **c)** mean of the maximum temperature of the warmest month, during the hydrological year, for the four years of the study (mean ± SD, n=9). Different lowercase letters indicate significantly different means.

Finally, it is important to stress that, overall, variation in trait mean (CWM) showed a higher dependency on short-term climatic fluctuations, and also on local environmental factors, than functional diversity. Conversely, inter-annual variations in functional diversity were more influenced by long-term climate, than CWM. This indicates that functional diversity might be more influenced by the climatic filtering acting on the trait regional pool over the long-term. It also suggests that functional diversity has higher resilience than CWM to short-term climatic fluctuations, as suggested by previous studies (Carmona et al., 2012), although this may depend on the particular trait being addressed (Carmona et al., 2015). This further reinforces the potential of functional diversity to be used as an indicator of climatic changes that persist through time, and to integrate the list of biodiversity-based indicators claimed by the United Nations Convention to Combat Desertification (UNCCD), and also by the UN Conventions on Biological Diversity (UNCBD), and on Climate Change (UNCCC), to track climate change impacts on dryland ecosystems.

8.8 Shrub encroachment management and climate change

Shrub encroachment is a matter of concern in many drylands worldwide, mostly because it affects the provision of ecosystem services e.g. related to livestock production, or may be detrimental for species with high economic and/or ecologic value (Caldeira et al., 2015), despite contradicting perspectives on its effect on ecosystem functioning (Eldridge et al., 2011, D'odorico et al., 2012, Caldeira et al., 2015, Maestre et al., 2016). The causes of shrub encroachment, which are known to diverge between different geographical regions, lacked scientific evidence from the Mediterranean Basin. Our work contributed to fulfill this knowledge gap. We found that, under low-intensity and similar land management, shrub encroachment was mostly driven by local topo-edaphic factors. Our results suggest that shrub encroachment is more influenced by the control that topo-edaphic factors exert on soil water availability at different soil depths, and less by variations on the total amount of precipitation. Yet, precipitation during summer had a negative influence, although weaker, on shrub encroachment, suggesting that precipitation seasonality might play a minor role. Precipitation during warmer periods favors water availability at upper soil depths. Under these conditions, herbaceous vegetation has a competitive advantage over shrubs, due to their shallower root systems. According to our results, in the Mediterranean Basin, climate change is not expected to promote shrub encroachment, in contrast with what previous works suggested (D'odorico

et al., 2012). A recent work addressing climate change effects on temperate drylands, points in the same direction as our findings (Schlaepfer et al., 2017). It is worth noticing that soil properties (e.g. texture, depth) may be seen as the result of long-term climate effects. However, these processes operate over longer time scales (Jenny, 1994), beyond the scope of our analyses, and of the time scales of predictions of shrub encroachment due to climate change. Our findings imply that management actions to reduce shrub encroachment should take into account topo-edaphic factors to be cost-effective. Methods involving alterations in terrain structure to change water and nutrient flow pathways and accumulation, are more likely to sustainably succeed, than the 'traditional' mechanical shrub clearing. Our work contributes to improve management actions to revert shrub encroachment in the Mediterranean Basin.

8.9 Is trait-based ecology being used in dryland restoration?

Desertification and land degradation consist of the loss of ecosystem functioning. The main means to reverse it is through active restoration. By providing a comprehensive overview of the current restoration practice in Mediterranean drylands, our work showed that trait-based ecology is still scarcely used in restoration practice. Many projects relied on the use of non-native species in restoration, thus disregarding the potential benefits of using native species and their functional traits to accelerate ecosystems functional recovery. Conversely, plant functional traits can be useful indicators of the functional recovery of 'restored' ecosystems (Hooper et al., 2005) and associated ecosystem services (Díaz et al., 2007, de Bello et al., 2010). They can also allow a better assessment and prediction of the sustainability and resilience of the restored ecosystems (Sterk et al., 2013), particularly under a climate change scenario (Suding et al., 2015). However, less than 25% of the projects used plant functional traits as indicators in restoration monitoring. Although most of the practitioners reported the recovery of ecosystem services as a restoration goal, the vast majority did not make any attempt to quantify ecosystem services in the 'restored' ecosystem. We identified the need for a better integration of trait-based ecology into dryland restoration practice. We provide indications on what is needed to improve and promote ecological restoration in drylands, including the need to foster the use of functional trait indicators in restoration monitoring and evaluation.

8.10 Future challenges

Our work contributed to advance the knowledge on plant functional response to desertification and land degradation due to aridity, and to understand its variation in space and over time. We developed a functional diversity-based indicator of ecosystem functioning loss due to aridity to be used at a global scale, and demonstrated the need of a better integration of trait-based ecology into dryland restoration. Despite the knowledge gathered so far, some future research lines can be drawn from this work.

Our trait-based indicator of aridity impacts on ecosystems needs to be extended and validated in other dryland regions. This is essential to confirm that the relationship we found between functional diversity and aridity is generalizable across geographic locations and taxonomic composition. Similar studies are being developed e.g. in Caatinga semi-arid ecosystem in northeast of Brazil, which will enable to address this need.

To a better understanding of functional diversity response to climate over time, we need to obtain and explore longer-term data sets; manipulative experiments in the field in addition to gradient analysis, e.g. using rainout shelters, can be a complementary useful way to further explore the relationships between plant functional diversity and climatic variation.

We need better proxies of soil water availability to plants with high spatial and temporal resolution, which integrate the effect of topography and of soil properties as modulators of the effect of climatic variability on plant communities. This would contribute to a better disentangling of complex interactions between regional and local environmental factors.

Other plant traits, beyond the ones we studied in this work, might be worth exploring, such has leaf life span, or clonal and bud bank traits, particularly on perennial species. Root depth and distribution using more detailed data should be further explored in future works.

Seed banks play a key role in species persistence in drylands. The study of their composition and longevity may help to better understand species mechanisms to deal with spatial and temporal heterogeneity in drylands, legacy effects, and to test existing ecological theories about interactions between seed traits and dispersion strategies.

The analysis of intraspecific trait variation along climatic gradients in drylands should help clarify its relative importance, at different spatial scales.

Variation in species or trait composition within and between communities (along space or over time) may be due to their loss or replacement and/or to changes in their abundance. The use of approaches based on the partitioning of taxonomic and functional diversity into α (within communities) and β diversity (between communities) in futures studies could be useful to better understand these changes at different spatial and temporal scales.

Scientific evidence gathered so far regarding the relationship between plant functional traits and specific ecosystem processes, derives mostly from manipulative studies on experimental plant communities. The examination of such relationships in the field could complement those studies and improve our understanding and their predictive ability.

The relationship between plant functional traits and ecosystem services is one of the main current challenges of trait-based ecology, and should be addressed in future research, particularly the upscaling of information from local plant sampling to regional and broader spatial scales (e.g. based on remote sensing data).

Despite recent efforts to develop adequate metrics to measure 'ecosystem functioning', with the development of 'multifunctionality' indices, existing approaches have limitations and should be further explored and improved.

Trait databases need more trait data and a better geographical coverage, so future studies should also contribute to this endeavor.

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